Chem Ed

EDITORIALLY SPEAKING

Research and Teaching in the Liberal Arts College was the theme of a ten-day conference sponsored by the Division of Chemical Education and supported by the NSF at the College of Wooster in June, 1959. Extensive data were compiled by questionnaires answered by 341 institutions. These were analyzed and summarized by a hard-working group representing 30 colleges. These conferees were all from the faculties of colleges whose records show that they have been most productive in terms of providing the baccalaureate training for large numbers of Ph.D.'s in chemistry. The complete report is in the final stages of preparation for publication and will be sent to every college president in the country and to the heads of chemistry departments.

The work of the conference was to discover what correlations exist between the presence of research activity on a college campus and the productivity of the institution. At the same time, the teaching conditions (instructional load, laboratory space, library facilities, equipment, etc.) under which chemistry departments operate were correlated as objectively as possible with the productivity of the college.

The criterion of productivity was a specific and delineating one: the number of alumni who attained the chemistry or biochemistry Ph.D. degree in the years 1936–56. Government reports supplied the statistical information. It shows conclusively that "smallness" or "liberal arts" are by no means the labels which can universally guarantee good training in chemistry. Of the 341 institutions surveyed, only 30 have produced 30 or more Ph.D.'s in the 20-year period; another 45 colleges have produced between 15 and 29 Ph.D.'s; another 100 have averaged fewer than one per year (5 to 14 total); the rest of the colleges each have produced 4 or fewer over the 20-year span, many none at all.

It is clear from the assembled data that there is a strong correlation between good teaching (assuming good teaching is responsible for students desiring and being able to go on for the Ph.D.) and research on the campus. No one claims that research *makes* good teaching. Rather it is obvious that the institution thus attracts both better faculty and better students. An oft-repeated comment, both by faculty and student respondents, was that the intimate contact between a professor and his student while they were making a parallel exploration was the best possible form of career counseling. Just having a professor's special project set up in the corner of a student laboratory fosters respectful curiosity and sets the stage for professional counseling at its height.

We cannot in this space summarize all the evidence supporting the established correlation. It is significant that the report points out that similar problems exist in all colleges, regardless of their productivity. These have been met with imagination and ingenuity by some and with resignation by others. The faculty-student ratio is nearly constant in all colleges but unreasonable contact-hour teaching loads are found only in the non-productive groups. The report speaks directly to college administrations on this and several other scores. It is possible to schedule at least one day a week for a professor to have uninterrupted time. There is real efficiency in being able to plan within an established budget. There is a saving in hiring secretarial and stockroom help instead of paying for housekeeping by the faculty. It is possible by judicious selection to have a library which will support investigation, not merely be a collection of encyclopedias.

Representatives of 18 foundations attended the conference. The increased mutual understanding between them and the professors was a most valuable by-product. The statistics showed undeniably the role that support of research plays in providing an intellectual atmosphere in which good teaching thrives. In the period 1951–56, 300 colleges received over $3 million from various sources. The 30 very productive colleges received 57% of this. Planners for the future, including college presidents, will find this suggestive.

Readers who are unable to secure access to the report may request copies from the co-directors of the conference: Harry F. Lewis of the Institute of Paper Chemistry, Appleton, Wisconsin or John D. Reinheimer, College of Wooster, Wooster, Ohio.

A CHEM ED SYMPOSIUM

Biochemistry in General Chemistry?

Introduction

The current trends in the teaching of general chemistry are toward making the program broader and more general—more representative of the various branches of chemistry. Those in attendance at sessions of the Division of Chemical Education at recent national meetings of the Society have heard speakers plead for more inorganic chemistry, more physical chemistry, more analytical chemistry, more surface chemistry, etc. The justification for such pleas has usually been twofold: first, that especially important developments have recently evolved from that particular branch of chemistry—developments which have or will have far-reaching significance in the advancement of human knowledge; and second, that most students in general chemistry courses—about 85%—do not take any more chemistry. For these persons, it is argued, the general chemistry course should present an overall view of the science of chemistry, emphasizing some of the principles, unique thought patterns, and methods of organization of knowledge that characterize the various branches of chemistry.

This symposium is directed toward biochemistry with the obvious implication that this too be included in the general chemistry program. Some teachers have been doing this for some time and a fair percentage of modern general chemistry texts include at least one chapter on this topic. However, the first reaction of many teachers is that this is too much—"We have to draw the line somewhere." If these teachers could see the tremendous impact several well-developed lectures on biochemistry have on students, if they could examine the syllabuses of modern *elementary* biology courses and see how much some fundamental organic chemistry and biochemistry would help students understand and retain certain important principles of zoology and botany, if they could realize that in presenting some biochemistry they are really saying to their students, "These are some of the chemicals and chemical processes which keep your bodies alive and healthy," then perhaps their second reaction to the inclusion of biochemistry in general chemistry would be more flexible than the first.

These are some of the reasons this symposium was planned. The authors of the papers which follow have all had considerable experience in teaching biochemistry to undergraduate students. All have an active interest in biochemical research. The topics were selected in an effort to blend modern important biochemical developments with material which could be successfully presented at the freshman level. However, the material is presented not as a teaching program or syllabus but as informative summaries.

Perhaps it would be useful to include here the sequence of lessons used in presenting biochemistry in one general chemistry course. One week—two lectures, a discussion class, and a laboratory experiment—is given to this topic. During the lectures three basic notions are stressed. These are: (1) the speed of biochemical reactions; (2) the specificity of enzyme reactions; (3) the complex and sequential nature of metabolic processes. The first notion is illustrated by conducting experiments on plants and animals using labeled glucose or phosphorus and by describing experiments such as that reported by Du Vigneaud in his paper on the synthesis of oxytocin in which he comments, "Approximately 1 γ of either the synthetic or natural material (oxytocin) given intravenously to patients induced milk ejection in 20–30 seconds.[1]" The specificity of enzyme reactions is illustrated by such examples as tyrosine decarboxylase which will decarboxylate no compound but tyrosine, or glucose-6-phosphatase, found in the liver, which catalyzes the hydrolysis of glucose-6-phosphate only. Enzymes such as prostatic phosphatase which catalyze hydrolysis of a wide variety of phosphates are also included. The complex and sequential nature of metabolic processes is illustrated by tracing the path of a piece of starch from the time it enters the mouth until the glucose is converted into carbon dioxide and water or stored as glycogen. It is obvious that all of the known steps in this sequence cannot be included but a discussion similar to that given by Walker, Asimov, and Nicholas[2] has been used with apparent success. Experiments with salivary amylase demonstrate simply but effectively some of the principles discussed.

One of the big stumbling blocks in the way of including topics such as biochemistry in general chemistry appears to be a feeling of inadequacy on the part of teachers most of whom have not been trained in this area. It is hoped that this symposium will help convince many teachers that, despite apparently complex nomenclature, certain principles of biochemistry are easily presented and readily grasped.

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[1] Du Vigneaud, V., Ressler, C., Swan, J. M., Roberts, C. W., and Katsoyannis, P. G., *J. Am. Chem. Soc.*, **76**, 3115 (1954), p. 3118.

[2] Walker, B. S., Asimov, I., Nicholas, M. K., "Chemistry and Human Health," The Blakiston Division, McGraw-Hill Book Co., Inc., New York, 1956, pp. 256–72.

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Enzymes and Metaphor

A classic experiment that often serves to start a course in high school general chemistry is one in which oxygen is prepared by the decomposition of potassium chlorate. The directions for conducting the experiment are explicit. The student does not simply heat potassium chlorate. Manganese dioxide must be added first. Without it, heat must be strong and gas evolution, nevertheless, slow. With it, heat may be gentle and gas evolution fast.

It is necessary to explain to the student that the manganese dioxide does not enter into the reaction, if only to prevent the student from liberating oxygen on paper by reducing manganese dioxide to metallic manganese. The function of manganese dioxide is only to accelerate the breakdown of potassium chlorate in some fashion that does not consume the manganese dioxide. Its mere presence is sufficient. It is a catalyst, and the process of influencing by mere presence is called catalysis.

This, left to itself (as it often is), can lead the beginner to a lifelong and unnecessary association of catalysis with mystery. The notion of influence by mere presence rather than by participation is uncomfortably like a kind of molecular psi force, an extrasensory perception on the part of potassium chlorate that the influential aura of manganese dioxide is present, or perhaps a telekinesis, a supernatural action at a distance on the part of the aloof but godlike manganese dioxide molecule.

Any unnecessary aura of mystery in science is undesirable since science is devoted to making the universe less mysterious, not more so. The fact that any student going on to industrial chemistry will be constantly involved with catalysis and that any student going on to biochemistry will meet with those exceptionally useful protein catalysts, the enzymes, makes this particular unnecessary mystery especially undesirable.

It is, naturally, impossible to stop an introductory course long enough to delve into surface chemistry, or into the cyclic formation and destruction of catalyst-involved intermediates, or into energies of activation. For one thing the students lack the necessary background for it and for another it is not necessary. All that need be done at the start is to puncture the mystery; time enough later to supply the rationale.

To do away with mystery, it is only necessary to offer the students common examples of how a reaction can be hastened by mere presence of an extraneous substance; examples that, on the face of it, do not involve witchcraft. In short, a student may be unready for physical chemistry, but he is always ready for metaphor.

Once given the metaphor, it will, if dramatic enough, be retained. If the student never proceeds beyond the elementary chemistry course, he will yet avoid in this one tiny respect the plague of mysticism; and a contribution will be made to the rational view of the universe, which is one of the ends of scientific thought. If the student proceeds into advanced chemistry courses where catalysis will crop up again and be placed on a firmer theoretical base, he will at least have his conceptual start and be able to approach the subject with greater confidence.

Catalysis by Metaphor

For instance, how can a catalyst influence a reaction by its mere presence? What is there in ordinary life that can offer an analogy to such an esoteric phenomenon? Suppose we make use of the "brick-and-inclined-plane" metaphor.

Instead of potassium chlorate breaking down and down and down, liberating oxygen; imagine a brick sliding down and down and down a gentle incline, liberating energy. Both are spontaneous processes but both need initial pushes. The potassium chlorate requires the encouragement of heat; the brick will require the push of a hand.

Suppose the incline on which the brick rests is a rough one, however, so that there is a great deal of friction between brick and surface. Despite gravitational pull and the helping hand, the brick stops quickly once the hand is removed.

Now suppose you were to coat both brick and incline with ice. The brick suddenly slides more easily. A gentle push to start it, or even, perhaps, no push at all, is necessary.

Now the ice itself does not push the brick; it does not increase the gravitational force; it does not supply energy in any form; it plays no active role at all. Its mere presence is enough. Nor need much be present; only enough to coat thinly those portions of brick and incline that come into contact. Nor is any of the ice used up in the process, ideally. When one brick has moved on, the ice is all there; another iced brick can be set sliding, and then another and another.

A catalyst is defined as a substance capable of accelerating a chemical reaction by its presence in small quantities, without itself undergoing permanent change in the process. Eliminate the word "chemical" and the ice coating an incline is a perfect catalyst.

An alternative analogy is the "writing-board meta-

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phor." Imagine a man with a pencil and paper and nothing else standing in the midst of a desert with only soft, shifting sand underfoot. The man wishes to write a note upon the paper.

The man knows how to write, he has the wherewithal to write with, and the wherewithal to write upon. Nevertheless he can write only the most fumbling note, one that is very likely undecipherable, and will almost certainly tear the paper in the process.

Now imagine him suddenly endowed with a smooth writing board of polished wood which will not itself take a pencil mark. How different is the situation!

The man undergoes no increase in his knowledge of writing. His instrument of writing remains as before, the pencil. The only object upon which he can write a note remains the paper.

Yet now his message can be written smoothly, clearly, and painlessly—all thanks to a writing board which hastens the process by its mere presence and is unchanged in the process. Both paper and pencil are somewhat used up and the man himself has expended some calories, but the writing board has suffered no significant loss. It can be used for an indefinite number of similar jobs. It is, in short, a catalyst.

Both metaphors serve, furthermore, to introduce the notion that catalysis is essentially a surface phenomenon; that a reaction is hastened (whether it is the sliding of a brick, the writing of a note, or, by extension, the breakdown of potassium chlorate) by the provision of a surface that is specifically suited for the activities involved in the reaction.

Later in the course, the student may be presented with the notion that catalysis hastens a reaction without, however, changing the position of the equilibrium point. In fact, if one started with pure products, rather than pure reactants, the reaction would be hastened in the opposite direction—and to the same equilibrium point. To the student who first encounters this fact, there may seem something diabolical about an inanimate substance that can pull in either direction as though it knew in advance where the equilibrium point was.

Yet it is simple to demonstrate that the catalyst is not pulling in two directions but in only one, if we go back to our brick-and-inclined-plane metaphor. Imagine a double incline in the shape of a shallow, blunted V, made of a rough, high-friction substance. Again a coating of ice will serve as catalyst, allowing the brick to slide. Observe though that it will slide down either slope of the V and in both cases end at the same point, the bottom.

If the top of one arm of the V is labeled "reactants" and the top of the other "products" and the whole viewed from directly above, it does seem that the catalysis works in either direction, stopping at a mysterious midpoint that seems no different from any other point.

View it from the side, though, and you see at once that the reaction is in one direction only—downward, in the direction of the pull of gravity. The ice-catalyst accelerates this one downward movement. The endpoint (or equilibrium) is the lowest point in the V, the point of least gravitational potential, and hence quite unique. Even the least intuitive student can see that the ice is actuated by no mysterious foreknowledge of the "equilibrium" position. The brick simply slides to the bottom.

The student should see at once, too, why catalyzing a reversible reaction does not change the equilibrium point and why hastening a reaction in a particular direction does not cause it to move further in that direction. Obviously coating brick and incline with ice to enable the brick to slide more quickly doesn't alter the position of the bottom of the V or allow the brick to go past this "equilibrium point" and climb half-way up the other side and remain there permanently.

Nor need the usefulness of metaphor be restricted to only the more elementary notions. Later in his career, a student will be told that a catalyst achieves its results by lowering the energy of activation.

In other words, there is some unstable intermediate compound formed by the reactants, which breaks up to form the products. This unstable intermediate compound requires the input of a comparatively large amount of energy, but until it is formed, no products will be found although the products themselves are not particularly high-energy. The entire reaction will proceed no more quickly than the unstable intermediate can be formed.

The catalyst, by stabilizing the intermediate, allows its formation with a smaller energy input. This hastens the rate of formation of intermediate and consequently hastens the reaction as a whole.

Often the energy of activation is represented as an "energy hump" between products and reactants. The enzyme is shown as lowering the hump and thus increasing the traffic over it. Make a highway out of this, with automobiles passing in either direction, and it is itself an interesting metaphor. However, it does not show how a catalyst can lower the hump and this can be done, dramatically, by means of the "shoelace metaphor."

Imagine a man who is standing in a muddy field of indefinite extent who finds he must tie his shoelace. Now there is no danger of his flopping down into the mud (something he does not want to do) as he stands there with this shoelace untied. Once his shoelace is tied, he is again in no danger. Both are stable positions.

During the process of tying his shoelace, however, he must either squat, bend, or raise his foot while remaining standing. In each of these alternatives, he significantly increases the risk that he will get part of himself muddy or that he may lose his footing altogether. He must therefore work very slowly and carefully through the unstable intermediate position.

If we imagine a whole series of men, all of whom must tie their shoelaces under similar conditions, serially, no one man starting till the one before has finished, the whole process will take a long time for completion, just because of the slowness of that unstable intermediate step.

Now supply one firmly-placed chair and allow the man to sit in it. Once sitting, a foot can be raised without loss of stability. The shoelace can be tied without danger and the man can rise. The chair is not only a catalyst (again serving its purpose by offering a suitable surface) but it is one that specifically serves to stabilize the unstable intermediate position. It

lowers the "energy hump" in a manner that can be clearly visualized.

Now a large number of individuals can tie their shoelaces, one after the other, much more quickly if the chair is used by each, than otherwise. By stabilizing the unstable intermediate position, the chair-catalyst hastens the shoelace-tying reaction.

Metaphor for Enzyme Catalysts

When a student is first introduced to enzymes, he meets those catalysts which are at once the most intimately involved with man and the most "mysterious." Yet, although protein in nature, enzymes share all the fundamental properties of catalysts generally. The brick-and-inclined-plane metaphor, the writing-board metaphor, and the shoelace metaphor all apply to enzymes as directly as to manganese dioxide.

But enzymes introduce additional refinements also. One way in which protein catalysts (enzymes) differ from the inorganic catalysts is that the former are vastly more specific. It is not unusual to have an enzyme capable of catalyzing but one reaction out of the uncounted numbers possible. Yet this need not be accepted as a sample of the sweet mystery of life. Even a very superficial knowledge of protein structure would show that it would be possible to build very complex surfaces out of protein molecules by varying the nature and arrangement of the amino acid components. The value of a highly-specialized surface over a generalized surface can be pleasantly demonstrated by an extension of the shoelace metaphor.

A chair is a chair, but there are chairs and chairs. An ordinary kitchen chair is quite adequate as a catalyst with which to accelerate the tying of shoelaces. But now imagine a specially designed chair with back, arm rests and foot rests that are motorized and capable of automated motion. As you sit down, your weight upon the seat closes a contact and up flies one of the foot rests, lifting your foot to just the right height. Simultaneously, the back moves forward, tilting you appropriately, while the arm rests move inward, bending your arms at the elbow and gently forcing your hands together. In the fraction of a second, and without effort on your own part, you have assumed the "shoelace-tying position." As soon as you are done, the chair flies back into position and a padded lever gently ejects you from the seat. It is now ready for another individual.

Obviously such a specially-designed chair would hasten the shoelace-tying reaction to a greater extent than the generally-designed kitchen chair could. It would also further stabilize that unstable intermediate position. Furthermore, such a specially-designed chair by the very virtue of its specialization becomes less useful for other purposes. Unthinkingly, a young man might attempt to use it in order that he might hold his best girl on his lap. The motion of the various portions of the chair may surprise him. Yet even though he might find those motions endurable under the circumstances and even pleasant, he would almost certainly be disconcerted by the final ejection, as would the young lady also in question.

And if you only intended to use the chair to read a newspaper, you would abandon it in disgust even before you were ejected. In either case, you would seek a generally-designed chair on the next occasion, or a chair specially-designed for girl-holding or paper-reading as the case might be.

In short, the specially-designed chair (enzyme) is at once a more efficient and more specific enzyme than the generally-designed one (inorganic catalyst), each characteristic almost necessarily implying the other.

Nor need we devise imaginary chairs to make the point. One can as well, if not as amusingly, use the notion of a barber's chair, a dentist's chair, a soda-fountain stool, or an electric chair and compare each with a kitchen chair to point out how a specialized surface at once increases efficiency and specificity.

The notion of specificity enters into the idea of competitive inhibition as well. An enzyme may specifically catalyze the oxidation of substance A, let us say. It will not catalyze the oxidation of different substance B, nor yet of similar (but not identical) substance A′, yet the presence of A′ will interfere with the normal functioning of the enzyme with respect to A, while the presence of B will not.

Here we can use that most familiar of all enzyme metaphors, the lock-and-key metaphor. An enzyme working on a specific substance, A, may be compared with a lock to which A is the key. Substance B, which is nothing like A, is a key with its shaft completely different in grooving from that of A. It cannot even be inserted into the lock. As far as the lock is concerned, the presence of B has no meaning.

But now you have a substance A′ which is similar to A. It represents a key with a shaft similar to that of A. Therefore, A′ can be inserted into the lock. However, the notches of A′ are not similar to those of A. Therefore A′ will not turn the lock. But it is *occupying* the lock. While it is there, though it will not turn, neither will it allow A to enter. The lock is temporarily useless or, if you prefer, the enzyme is inhibited.

The student will not only meet enzymes, he will meet groups of enzymes. A day will come when he will find that compounds within the body yield energy by having their hydrogen atoms transferred two at a time to molecular oxygen. Water is formed and energy is released. Most of the energy released is stored in the form of high energy phosphate esters, about three of these being formed for each pair of hydrogen atoms transferred. The hydrogen transfer is accomplished in a number of steps, with the hydrogen being passed from position to position, bucket-brigade fashion, each step being catalyzed by a separate enzyme and only in the last step is it finally transferred to oxygen.

Why the series of enzymes? Would it not be better and simpler to combine the hydrogen atoms directly with molecular oxygen in a single step and use but a single enzyme to catalyze the reaction. As usual, we can find a metaphoric answer—the staircase metaphor.

Suppose it were necessary for a man to move from the fifth story to the ground floor and store the gravitational potential thus given off by winding up three clocks. He could do this by pulling chains as he passed each clock and thus raising their weights by the pull of his own weight as he moves downward.

Now he can go from fifth floor to ground level by means of five flights of stairs (a multi-enzyme system) and can, in the process move relatively slowly, seize the clock chains surely, and pull them smoothly.

The man might also go from fifth floor to sidewalk level by jumping over the banister and down the stair well (the one-step method.) He would get to the sidewalk more simply and more quickly and lose gravitational potential as surely as by a stately progress down the stairs. However, he will find it difficult to snatch at the clock chains as he passes. He will release energy, but will not store any.

Again, the stair method of going from fifth floor to ground level is reversible. One can move back up those same flights of stairs from ground level to fifth floor without a prohibitive expenditure of energy. However, having jumped down in a single bound, one cannot, alas (even supposing one is in a position to try) bound back to the fifth floor in a single leap.

Similarly, a multi-enzyme reaction, in which each component reaction involves a relatively small free-energy change, allows a more efficient energy storage and is, at the same time, more easily reversible and hence more efficiently controlled by the body. The large free-energy change of the one-step method (although apparently this is the simpler alternative) makes it difficult to store energy efficiently and still more difficult to reverse at need.

Metaphor: Catalyst for Learning

These metaphors are not intended to be an exhaustive list or even to be samples of the best possible; they are merely those which appeal to my own imagination, and each teacher, of course, is free to make up those which best suit his own personality and ways of thinking. It is not the metaphors themselves, but the principle of metaphor that I value.

It remains only to add that the judicious use of metaphor in the introduction of new concepts, generally, is not only an aid to understanding but adds also to the interest and pleasure of the course. Naturally, we all feel that science is its own reward and requires no artificial sweetening to be stimulating and fascinating, but unaccountably, as we all know, many students fail to see this.

Metaphor, then, by way of both reason and psychology, is itself a catalyst. By its mere presence and without actually increasing the scientific content of a course, it hastens the process of learning and is not used up thereby.

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The Functioning of Vitamins and Hormones

Since chemistry is a live subject, it can be taken for granted that the introductory courses and the textbooks which will be used ten years hence will be different from what they are now. The following decades will bring about further changes.

This symposium is dedicated to the idea that such introductory courses will be altered to include more rather than less material dealing with the chemistry of living things—biochemistry. This seems like a reasonable idea because biochemistry has advanced very rapidly in recent years. As judged by almost any criterion, it is ten times as prominent now as it was a few decades ago.

Among the subjects which inquiring students will want to learn about early in their chemical training are the vitamins and hormones. Unless they do so, they may be left behind even by their nonchemically oriented associates. Particularly may this interest be stimulated if students realize, as they will gradually come to do, that the functioning of vitamins and hormones are matters that touch their own individual lives and that diverse problems result from the fact that body chemistries are far from uniform.

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Metabolism is the sum total of everything chemical that happens in our bodies. Vitamins and hormones are both concerned in metabolism, and we will attempt to discuss in a simple manner the question "How?"

A prime distinction between vitamins and hormones is that vitamins are of exogenous origin (from the outside), while hormones are produced within our bodies—endogenously. Unless an agent is an exogenous one it cannot be called a vitamin, and unless it is endogenous it cannot be designated a hormone. There are a few examples of substances which may be both of exogenous or endogenous origin. Vitamin D is an example. It may be furnished entirely in the food and is hence classed as a vitamin. However, it may be produced in our skins by irradiation (significantly with ultraviolet light) of sterols which are also produced endogenously so that in the presence of plenty of sunlight it may not be needed in the food at all and hence does not function like a vitamin.

The thyroid hormone is a conspicuous example of a recognized hormone which may have a double-barrelled origin; it is produced adequately in the thyroid glands of healthy bodies, but it may also be readily obtained by consuming the thyroid glands of other animals. "Normally," we say, it is of endogenous origin.

An important point to be stressed with chemistry

students who are interested in learning something about vitamins and hormones is that neither term is the designation of a *chemical* grouping or classification. Vitamins are not like each other and do not "belong together" chemically. Hormones as a group also fail to resemble each other chemically. The classification together of certain substances as vitamins is based upon biological rather than chemical grounds—vitamins are always nutritionally important and effective in small amounts. Similarly the classification of substances as hormones is based not on chemical similarity but on biological similarity—they are all produced endogenously and in small amounts bring about diverse biological effects.

There are produced in the body approximately sixty different recognized hormones. Of these more than half are steroid in nature and contain only carbon, hydrogen, and oxygen. Many of these arise in the cortex of the adrenal gland. Other hormones with relatively low molecular weight (170–80) such as adrenaline and noradrenaline contain nitrogen in addition to carbon, hydrogen, and oxygen. Triiodothyronine (mol. wt. *ca.* 650) from the thyroid gland contains carbon, hydrogen, oxygen, and nitrogen and one additional element, iodine. Several hormones, are polypeptides—glucagon, oxytocin, vasopressin—and have molecular weights of the order of 1000. The thyreotropic hormone and insulin are relatively low molecular weight proteins with molecular weights 10,000 to 12,000. ACTH is likewise protein in nature and while it appears to have a molecular weight of 20,000, it can dissociate into smaller active fragments. Several other protein hormones, all arising in the anterior portion of the pituitary, have molecular weights in the range of 32,000 to 100,000: lactogenic hormone, the luteinizing hormone, the growth hormone, and the follicle-stimulating hormone. The parathyroid hormone appears to top the others in molecular size since its molecular weight is said to be 650,000.

The vitamins are also a motley group of chemical substances. There are about sixteen independent ones each capable of performing its own functions. Several forms exist in a number of these specific cases. The vitamins possess molecular weights from about 180 up to 1400. One hydrocarbon—carotene—(two elements only) can perform the functions of a vitamin. At the other end of the scale so far as complexity is concerned is vitamin B_{12} which contains six different elements. Vitamins are all organic compounds but are highly diverse in that they may contain straight or branched chains, rings—alicyclic or aromatic. Vitamin B_{12} structurally contains eight different rings, the majority heterocyclic.

The Enigma and Challenge of Metabolism

The relationship between hormones and metabolism is still very obscure. That they may have a profound effect is quite evident. The thyroid hormone as is well-known speeds up total metabolism, and some of the other hormones work subtly to modify metabolic activities locally in specific tissues and cells. Among the hormone-like substances are the neurohumors, acetyl choline and sympathin, which are released by nerves and may, for example, induce local muscular action and modify local metabolism. Excitement, for example, may destroy one's appetite for food; this must be accompanied by metabolic changes in the appetite-regulating centers. Worry can cause ulcers (in susceptible individuals); this doubtless happens through the agency of nerves and the local metabolic changes induced by agents which the nerves release. Emotional states are often greatly affected by hormones; these must involve metabolic changes in nervous tissue. A striking example of this is the promotion—to a high degree— of the mothering instinct in virgin rats when they are administered the lactogenic hormone.

The very large field of the relationship of hormones to metabolism, embodying attempts to answer the question of precisely how hormones work chemically, is for the most part a blank page in our knowledge—a page that will doubtless be gradually filled in, in the decades to come.

The relationship between the typical "fat soluble vitamins" and metabolism is likewise very obscure—comparable to that between the hormones and metabolism. Vitamin A is a partial exception in that it enters into the make-up of the conjugated proteins, rhodopsin, porphyropsin, and iodopsin, which are pigments essential to the process of vision. Vitamin A is, however, essential in other parts of the body, having nothing to do with vision, but how it functions in these other situations is not known.

Vitamin C, ascorbic acid, should have been the vitamin first to be discovered because clear-cut evidence for the existence of something of the kind existed over a hundred years ago. At the time not many people were chemically oriented in their thinking sufficiently to grasp the available evidence. In spite of its relatively long history the functioning of vitamin C remains an enigma—and a challenge. For some reason which is quite obscure, a deficiency of this substance in the body leads to a failure to manufacture intercellular cement. This may be only one of the reasons for its existence, but it is a conspicuous one. Another unexplained fact in connection with ascorbic acid is its relative richness in the adrenal glands.

Many of the so-called "B vitamins" are definitely parts of enzyme systems, and their relationship to metabolism is thus relatively clear. Enzymes cannot be made in our bodies unless the potentialities to produce them are present. The specific potentialities arise from the genes, which are handed down from generation to generation. In order for the enzymes to be made it is essential that the raw materials for their construction be supplied. These raw materials include the amino acids, the minerals, and the various B vitamins.

How the various "B vitamins" function in enzyme systems is a very active field of investigation and each year new significant links in the chain of evidence and information are forged. It is already clear, however, that we must be supplied with each of the B vitamins continuously in our diet because each furnishes raw materials for the production of some necessary catalyst. Thus with respect to these "B vitamins" our knowledge is much more explicit than it is with respect to the functioning of the other vitamins and the larger group of hormones.

One important point of view which needs to be emphasized—particularly with respect to the "B vitamins" about which we have more knowledge—is that they are not needed merely by the body as a whole; they are needed by every living cell and tissue. Every cell and

tissue has its functions to perform and every cell and tissue needs complete nourishment so that the catalysts will be active and cellular metabolism healthy and resistant to the mild poisons (by-products of metabolism) which are likely to be encountered.

One significant fact which certainly holds for most of the "B vitamins" and perhaps for some of the others is their universal presence in every kind of cell. This does not mean, however, that they are needed in equal amounts by all kinds of cells; indeed we know that when all the tissues are healthy the concentrations of specific vitamins in some tissues are much higher than in others.

During the process of "cell differentiation" the different types of cells produced—all from the original fertilized egg cell—vary greatly in size, shape, composition, and specific metabolic activities. This means that they must have quantitatively different needs for the raw materials from which they build their metabolic catalysts.

A knowledge of the functioning of vitamins thus involves a comprehensive picture of the metabolic activities of the numerous kinds of cells and tissues and a recognition of the fact that a lack of these fundamental raw materials may cause malfunctioning in any tissue of the body. Extreme lack such as is observed in classical deficiency diseases—beri beri, for example—results ultimately in gross malfunctioning of nervous tissue and the production of paralysis. Long before the paralysis is observed, however, cells and tissues all over the body have been suffering from a mild lack. Any and every cell may have its metabolic activity hampered because enzyme building has been blocked. These considerations make it possible to appreciate the fact that any specific B vitamin (these are universally needed by cells) may be "good for what ails you" even though "what ails you" may be any one of a number of entirely different disorders involving any one of a large number of types of cells and tissues.

Aside from satisfying our innate curiosity, one of the prime reasons for understanding the functioning of hormones and vitamins is that we may apply this knowledge to the better management of the health of our own bodies. As time goes on we will learn more and more about these natural agents and will be able to conserve and use them far more intelligently in maintaining our own health.

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Acceleration of Biochemical Research by Recent Analytical Developments

One of the remarkable features of recent scientific development has been the tremendous expansion of biochemical research. Progress has been breathtaking in rate and in extent of the vistas opened. Many reasons could be cited for this explosive advance but certainly one of the most important was the nearly simultaneous evolvement about a dozen years ago of a series of powerful analytical tools. The development of several kinds of chromatography and the widespread availability of isotopes as well as associated counting equipment permitted experimentation in areas that previously could not even be explored.

A very large fraction (perhaps half) of the current biochemical research simply could not have been carried out ten to fifteen years ago even if it had been conceived. At that time the biochemist lacked, by several orders of magnitude, the sensitivity and precision of measurement required to obtain necessary answers.

Consider for a moment the enormous analytical problems commonly faced by the biochemist. He may need to know precisely how much of each of a half-dozen different compounds is present in some solution even though their concentration may be only 10^{-3}, 10^{-4}, 10^{-5} M, or even less. Further, the compounds in which he is interested may be acidic, neutral, or basic. Some may be organic, some metallo-organic, others inorganic. They may be volatile, or nonvolatile. They may be closely related members of an homologous series or even isomers of a given compound. The situation is often still further complicated by the presence of a hundred or more additional unwanted compounds, some of them in macro quantities. As a final complication, the biochemist frequently finds it a very difficult task to obtain as much as one milliliter of the original juice which he wished to analyze.

Five kinds of chromatography (paper, solvent partition, ion exchange, adsorption or column, and gas-liquid) and isotopic tracers now make possible really quite elegant analyses even under the extreme conditions enumerated above.

The purpose of this paper is to present in highly simplified fashion the basic principles of these analytical developments so important to biochemistry. It is hoped that the level of presentation is suitable for use in general chemistry instruction. For example, when the subject of equilibrium is under discussion, some of the material on chromatography might be useful to illustrate the application of equilibria to analytical and biochemical problems. The use of biochemical examples serves the further purpose of reminding the undergraduate

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student of the existence of biochemistry. Since formal instruction in biochemistry is so largely at the graduate level, it is chiefly through such incidental allusions that the undergraduate student of chemistry develops any appreciation of the scope of biochemistry.

Chromatographic Methods

Paper Chromatography. Paper chromatographic separations involve the distribution of solutes between two solvent systems on a paper support. In a simplified model, the paper may be regarded as a continuous sheet of water (although the water has some odd properties) over which some solvent is flowing. If a solute is dissolved in either phase, an equilibrium is reached or approached with respect to the relative amounts of solute in the two phases. This situation is analogous to that in a separatory funnel or a liquid-liquid extractor. However, there is an important difference in that the solutes in a paper chromatogram are not free to diffuse or be convected throughout the water or solvent phase but, rather, stay in a discrete spot of relatively high concentration even when moving up or down a paper. Thus, fresh solvent flowing over water (paper) with high solute concentration will pick up some of the solute as equilibrium is approached. This solvent, now enriched in solute, will pass along the paper as the solvent ascends or descends until it is over an area of paper (water) containing less than the equilibrium amount of solute. Equilibrium will be approached at this new position by a movement of solute from the enriched solvent back into the paper. The original solute spot on the paper has been impoverished in solute. Thus, there has been a net movement of solute in the direction of solvent flow through an infinite series of paper to solvent to paper . . . . etc. movements. Solvent systems can be found which produce different rates of movement of two or more solutes, thus achieving a separation. The amount of solute required is measured in micrograms, and twenty or more chemically similar solutes can be separated and identified on one paper chromatogram. Semi-quantitative estimations of amount of separated solutes can also be made.

Solvent Partition. The solvent partition methods are very similar in principle to paper chromatography but may achieve the partition of solute between solvents on some inert support such as hydrated silica gel or even in a large series of tubes which are, in effect, modified separatory funnels.

Ion Exchange Separation. The use of ion exchange to deionize or soften water will be familiar to nearly every student of general chemistry, although the broad usefulness and theory of the process will be unknown. Perhaps the most simple theoretical explanation would be to regard the ion exchange substance as a bed of insoluble, nonreactive (though perhaps porous) material to which are attached more or less loosely some cations (or potential cations) in a cation exchanger or anions in an anion exchanger. As a solution of cations passes through a column of cation exchange material (often called resin) the cations in solution will have a certain tendency to displace the ions already on the exchange column. If the rate of flow of solution is slow enough, an equilibrium between the concentrations of a cation in solution and the concentration on the resin will be established. The distribution at equilibrium will be a function of the total concentration of the ion, the concentration and type of other cations, and the position of the given cation in a kind of activity series with respect to exchange ability on the particular exchange material being considered. There are other normally controlled variables such as temperature which affect the position of the equilibrium. In actual practice one seldom operates a column at a flow rate slow enough to permit attainment of true equilibrium. Rather, equilibrium is approached but not reached before new solution reaches the area. Thus, rate of flow and, of course, rate of approach to equilibrium are additional important considerations in the usual operation of a column for ion exchange separation. The movement of ions down an ion exchange column under the influence of an eluting solution is quite analogous to the previously described movement of a solute spot along a paper chromatogram. As the eluting solution reaches a region of the column containing adsorbed ions, some of the eluting ions replace some of the adsorbed ion, and equilibrium is approached. The flowing solution, enriched in the previously adsorbed ion species and impoverished in the eluting ion species, then passes on until it reaches a region of the column containing less than the equilibrium concentration of adsorbed ion. In an approach to equilibrium, some of the dissolved, previously adsorbed ion then goes back on the column. Thus, in an infinite series of "leapfrogging" motions from column to solution to column, etc., there is a net movement of the adsorbed ion down the column. Through proper choice of eluting ion and concentration thereof, two or more adsorbed ions can be made to move at different rates down the column and thus can be separated.

Adsorption or Column Chromatography. The movement of adsorbed solutes down an adsorption column bears considerable resemblance to that just described for the ion exchange column. The types of materials best separated by the two methods, as well as the forces involved in adsorption and elution, however, are different. In fact, adsorption chromatography permits separation of nonionic substances. Physical forces hold the solute more or less tightly to the adsorbent solid, such as alumina, with which an adsorption column is packed. Tightness of adsorption and ease of elution is related to the polarity of solute and eluting solvent. Usually, the more polar the solvent, the more powerful eluting agent it will be. Thus, commonly, one needs to take great care to have adsorbent, glassware, solvents, and solutions very dry at least in the first stages of operation of such a column. Otherwise, the presence of water will tend to elute the column, causing solutes to run down the column at an uncontrollably fast rate. In a properly operating adsorption column, there is a sequence of adsorption, elution, readsorption, re-elution, etc., similar to that observed in the other chromatographies.

Gas-Liquid Chromatography. For the limited number of heat-stable, volatile compounds important to biochemistry, the recently developed gas-liquid chromatography is a powerful analytical tool. In this process, a material adsorbed on a solid (similar to adsorption chromatography) or dissolved in an inert liquid on the solid (similar to paper or solvent partition chromatography) is moved by a combination of elevated temperature and elution with a stream of inert gas. When a solute is

finally swept off the end of the column by the inert gas, its presence in the moving gas stream is quantitatively and/or qualitatively determined by various physical means such as gas density, thermal conductivity, etc. This procedure has been enormously useful to the organic chemist but much less so to the biochemist because many biochemical substances would be either nonvolatile or unstable under the conditions employed.

Common Theory of the Chromatographies

A very general, unifying theory common to the five kinds of chromatography can be presented. In all kinds of chromatography there is a stationary compartment, originally containing solutes, and a moving compartment (Figure 1). Separable solutes *A* and *B* reach or approach an equilibrium between the two compartments, but with differing rates or final equilibrium values. The moving compartment after sufficient contact with the stationary compartment to become enriched in solutes, then moves on until it is opposite a region of the stationary phase containing less than the

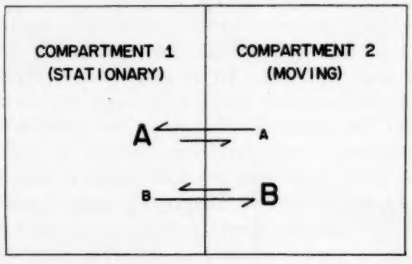

Figure 1. Equilibria in chromatography.

equilibrium amount of solute. Equilibrium, or an approach toward it, causes the solutes again to partition between the moving compartment and stationary compartment. Thus, there is a net movement of solutes forward. In the example illustrated in Figure 1, the equilibrium value (or rate of approach to equilibrium) for solute *B* is more in favor of the moving compartment than is the value for solute *A*. Thus, *B* is more readily eluted, and less readily reabsorbed than *A*. Therefore, *B* will move farther and faster than *A* and a separation will be achieved through passage of a series of successive "waves" of the moving compartment over the stationary compartment. Forces holding *A* and *B* to the stationary phase as well as forces driving the solutes toward equilibrium in the moving compartment may differ widely in the several kinds of chromatography, but the general relations described hold in all cases.

Isotopic Tracers

Biochemists are now able to trace the path of a substance through an organism or through a sequence of chemical reactions by the use of radioactive or separated stable isotopic tracers. Frequently this is the only feasible means of study of the problem at hand and fortunately great sensitivity of measurement is usually possible.

Tracers are usually used in one of two ways: either complete recovery of the isotopic tracer compound is attempted at each step with minor regard for chemical purity or highly purified compounds are separated and their specific activity (amount of tracer isotope per unit weight) is determined, with minor regard for losses of isotope during purification.

Examples might help to clarify these two approaches. Scientists studying the fallout problem are interested in the fate of a particular isotope, e.g., Sr^{90}, when it is given to an animal. Amounts of Sr^{90} so small that they are completely beyond the range of chemical detection are given to animals and at frequent intervals samples of bone, blood, many other tissues, and excreta are taken for analysis, i.e., for determination of the amount of radioactivity from Sr^{90} present. From such data, the radiation dose in each tissue can be calculated and an estimate of the probability of tumor formation, etc., can be made. Complete recovery of all administered isotope is required in order to account for all possible radiation dose. In such analyses normally the strontium is not isolated from the tissues but is very crudely separated by such means as ashing to drive off the compounds of carbon and hydrogen. The radioactive strontium is then determined by counting with a Geiger counter or other suitable device.

For numerous illustrations of the other major method of using isotopic tracers, one needs to look no farther than several of the other papers in this symposium. Much of biochemistry is now concerned with how a given compound, A, is converted into another compound, B. It is useful to know whether this involves a direct conversion of A to B; an indirect one via compounds C, D, and E; or possibly some other indirect conversion such as via compounds X and Y; or even all three mechanisms operating at once. Isotopically labeled compound A is given to the organism or biochemical system being studied, and then after the reaction has proceeded for an appropriate time, the various labeled compounds that have been synthesized are carefully separated, identified, and purified. The specific activity of each compound is measured and compared with that of the starting compound. The higher specific activities usually indicate more direct relationship to the starting compound. From the pattern of specific activities and his knowledge of biochemistry, the biochemist can then make a shrewd guess as to the mechanism of the reaction from A to B. More importantly, he can devise subsequent, perhaps definitive, experiments with isotopes which will prove the mechanism. In nearly all such studies, absolute purity of the separated compound is essential for comparison of specific activities, although considerable losses of product during purification are normal and completely satisfactory. One is concerned with the concentration of tracer rather than the absolute amount.

To obtain a crude quantitative estimate of the frequency of use of the several techniques described, a tabulation was made from the February, 1959, issue of the *Journal of Biological Chemistry* (see Figure 2). When more than one of the above techniques was used in one research it was recorded under each technique employed. Isotopic tracers were used in 40% and paper chromatography in approximately 25% of the researches. Some form of chromatography was used in approximately 50% of the studies. It can be seen that there is no entry for gas-liquid chromatography. This is because there was no use of such chromatography recorded in this issue.

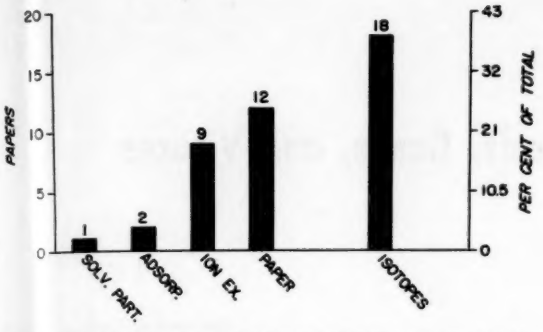

Figure 2. Relative frequency of analytical techniques employed in biochemical research (Feb., 1959, issue of *J. Biol. Chem.*).

Typical Applications to Biochemical Research

Currently, in the author's laboratory there is the necessity of determining whether certain plants secrete any organic substances via their roots into the soil. Plants will be grown in an atmosphere containing radioactive carbon as $C^{14}O_2$ so that the organic compounds of the plant slowly become labeled with the C^{14}. If any of this labeled material is secreted through the roots into the soil, or in this case, into the sterilized sand and hydroponic solution, it should be possible to detect its presence by the radioactivity. In preliminary studies, extracts of the solution around the roots and also extracts of the roots themselves were freed of nearly all of the inorganic salts by passage successively through an anion exchange column and then a cation exchange column. The effluent from these columns which contains any nonionic solutes can be examined for radioactivity. Careful elution of the columns has yielded a series of fractions, some containing organic substances, some not. The organic samples were concentrated by suitable means and a small aliquot of each was subjected to paper chromatography. The paper then can be examined for the presence and position of radioactive spots by either scanning it with a Geiger counter or by placing the paper next to an X-ray film for a suitable time. Any radioactive spots will show up as dark spots on the developed X-ray films. These may be matched with the chromatogram and the position of such spots can be plotted on the chromatogram. Up to this point all of the separations can be carried out with almost no knowledge of the chemical nature of the compounds being separated. Since knowledge of the chemistry of some of the compounds is essential to the research it is necessary to separate and purify substantial quantities of the compounds for identification purposes. Such quantity separations on the types of solutions in this study are best carried out by solvent partition chromatography of already partially purified ion exchange column effluents or eluates.

In certain cases, we have made an initial purification by adsorption in passage through activated charcoal. Thus, in this one fairly simple piece of biochemical research nearly all of the analytical techniques referred to are being used. An examination of the biochemical literature, or even of the other papers in this symposium will reveal that such a situation is quite common.

It would be most helpful for biochemists in graduate training if, as undergraduates, they had become thoroughly grounded in the principles, theory, and applications of these modern techniques. By the use of suitable biochemical examples as early as the general chemistry training, the students could be given some notion of the scope of biochemistry even without any formal undergraduate course in the field.

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Training and Experiments in Radioactivity

A collection of thirty-six papers from THIS JOURNAL related to training and experiments in radioactivity has been prepared by Nuclear-Chicago. The 114-page volume of carefully selected material is attractively prepared. One copy is available free upon request from Nuclear-Chicago Corporation, 333 E. Howard Avenue, Des Plaines, Illinois. Orders for multiple copies will be filled at a charge of $1.00 per copy.

Paul C. Aebersold, Director of the AEC Office of Isotopes Development comments:

"The collection of course material oriented toward both college and high school levels of instruction in this publication should contribute materially toward making training opportunities available to those students who will be tomorrow's scientists and industrial engineers. . . . Students at all levels will benefit by the opportunity of using tested radioisotope techniques taken from the diverse experiences of the contributing authors."

The Editors of THIS JOURNAL are gratified to have material from our pages thus made available as an important document. Years ago a former editor coined the phrase, "The JOURNAL OF CHEMICAL EDUCATION is a living textbook of chemistry." We are pleased that others think so, too.

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Nucleic Acids, Genes, and Viruses

In recent years truly remarkable progress has been made toward the goal of explaining in chemical terms the all-important biological phenomenon of heredity, the genetic transmission of characteristics from cell to cell. Although many of the details remain to be established, the fundamental processes now seem clear. The major purpose of this paper is to review in general terms our present knowledge of and current thinking concerning the chemical basis of cellular inheritance.

The primary concept of genetics is that of the gene, the unit of inheritance postulated to exist on the basis of much biological data. The gene is defined in terms of function, and for our purposes it must be kept in mind that a gene must serve two roles.

The first of these roles results in the expression by the cell or more complex organism of a specific characteristic. The gene is pictured as providing the cell with certain information, information which the cell uses to create a certain effect. For each such effect or characteristic exhibited by a living cell, at least one unique information unit, one kind of gene, must be present.

The second role of the gene is to supply the information needed by the cell to bring about the duplication of that gene prior to cell division so that each daughter cell receives an information unit for use in its own growth and development.

Another concept of genetics is that of mutation, the alteration of a gene with a resulting alteration in the inherited characteristic. If the gene alteration or damage is great, complete loss of the related characteristic may occur. If the alteration is minor in nature, a slightly modified characteristic may be expressed. In either event the change in the gene becomes part of the inheritance of the cell and its descendants. Upon cell division, the altered gene is duplicated in its new altered form or is not duplicated at all and is lost.

These biological considerations should evoke a number of questions in a chemist's mind. What is a gene in terms of chemical structure? What are the chemical mechanisms leading to the expression of a distant characteristic by a gene or group of genes? What is the chemistry of gene duplication?

It is becoming quite certain that the answer to the first of these questions is that the gene, the postulated functional unit, is a molecular unit, a complex molecule of a type termed nucleic acid. Some of the more conclusive evidence for this statement will be given later. At this point, however, the chemical nature of the nucleic acids will be examined, for it must be realized that biological function is a reflection of chemical structure.

Presented as part of the Symposium on Some Aspects of Biochemistry of Particular Interest to General Chemistry Teachers before the Division of Chemical Education at the 135th Meeting of the American Chemical Society, Boston, April, 1959.

Chemical Nature of Nucleic Acid

The nucleic acids are a group of very large compounds with molecular weights in the range of about 10^6 to 10^7. They are linear or chain-like molecules, and they may easily be hydrolyzed into a number of much smaller units. A nucleic acid molecule may accordingly be considered to be a polymeric compound. Biochemists, however, prefer to call such compounds macromolecules, for the term polymer implies to many chemists a random arrangement of the monomeric units or a simple repeating structure. Each biological macromolecule, on the other hand, is composed of a variety of monomers linked in a precise, invariant sequence. Each nucleic acid molecule is differentiated from its kin by the relative proportions of the different monomeric units and by the particular sequential arrangement of the units in the macromolecular chain.

The monomeric units of the nucleic acids are called nucleotides and a nucleotide chain or nucleic acid molecule is, therefore, sometimes called a polynucleotide. Each nucleotide is composed of three subunits, a phosphate group, a sugar, and a nitrogen-containing ring compound. The nucleic acids are divided into two main groups on the basis of the sugar found in the nucleotides. For one of these classes, the ribonucleic acids, the sugar is D-ribose. For the other, the deoxyribonucleic acids, the sugar is 2-deoxy-D-ribose. Ribonucleic acids are commonly abbreviated as RNA; deoxyribonucleic acids as DNA.

The nitrogenous compounds of the nucleic acids, commonly termed the nucleic acid bases, are N-heterocycles of the types known as purines and pyrimidines. Each nucleic acid molecule, regardless of kind, contains with minor exceptions monomers of four types, two purine nucleotides and two pyrimidine nucleotides. These purine and pyrimidine compounds are amino and keto derivatives of the parent ring systems. The same two purines, adenine and guanine, are found in both RNA and DNA, as is the pyrimidine, cytosine. The fourth base, however, is uracil for RNA and thymine, 5-methyl uracil, for DNA.

A typical nucleotide is pictured in Figure 1. Note that the sugar is linked in glycosidic union with a nitrogen atom of the pyrimidine ring compound, here uracil, and that the phosphate group is bound in an ester linkage to one of the alcoholic hydroxyl groups of the sugar.

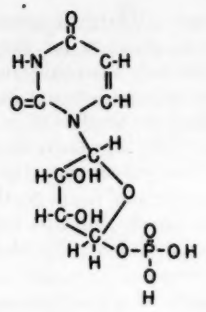

Figure 1. Uracil ribonucleotide.

The nucleotides are joined to each other by the formation of a second ester linkage involving the phosphate group of one nucleotide and a hydroxyl group of another nucleotide. The resulting macromolecule is essentially a chain of alternating pentose and phosphate units, linked by means of phosphodiester groups, with the purine and pyrimidine bases projecting from the chain.

In the long polynucleotide chain, there is no known restriction as to the possible arrangements of the units. A single nucleic acid molecule of known nucleotide composition may, then, be any one of an almost unlimited number of possible isomeric forms with respect to nucleotide sequence.

Little is known concerning the fine details of the structure of the RNA macromolecule, but a great amount is known for DNA. The difference resides in the difficulty of preparing RNA in the crystalline or ordered state. This has been accomplished for DNA, permitting the application of X-ray diffraction techniques. These techniques have clearly demonstrated that the DNA molecule consists of two polynucleotide chains interwound in helical fashion around the same axis. The resulting structure is pictured in diagrammatic fashion in Figure 2. It may be seen that the deoxyribose-phosphate sequence of each chain occupies an exposed, exterior position, forming two helical ribbons. The bases are planar structures; in DNA the planes of the bases are all parallel to each other and perpendicular to the axis of the double helix.

The most significant aspect of this structure is the means by which the two chains are held together to give the long, thin, relatively rigid molecule. This is accomplished by hydrogen bonds involving the amino and oxy groups of each of the pairs of bases which lie opposite each other. From stereochemical considerations and from the nature of the potential H-bonding groups of the various bases, it can be shown that only certain base pairings are feasible, pairings which involve adenine and thymine on one hand and guanine and cytosine on the other.

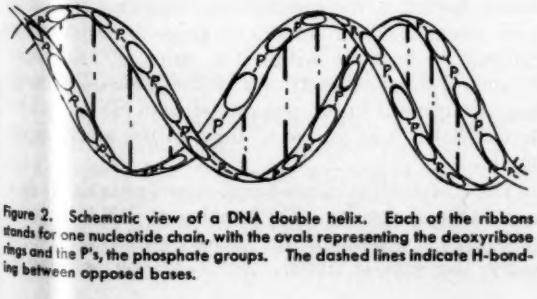

Figure 2. Schematic view of a DNA double helix. Each of the ribbons stands for one nucleotide chain, with the ovals representing the deoxyribose rings and the P's, the phosphate groups. The dashed lines indicate H-bonding between opposed bases.

The nature of the H-bonding between adenine and thymine is depicted in Figure 3. Similar interactions occur with guanine and cytosine. Relatively strong interaction is provided through the formation, in effect, of a 6-membered ring. This interaction, multiplied by the number of base pairs in a DNA unit, gives rise to a high, total force holding the individual chains in place. The DNA structure, therefore, is not that of a single molecule in the sense of covalent bonding, but that of a specific aggregation of two molecules. The binding, however, is of such strength and of such specific nature that the unit is commonly spoken of as a molecule.

It should be noted that the specific base pairings which have been indicated are supported by much analytical data demonstrating that, within experimental error, every DNA sample of a living organism

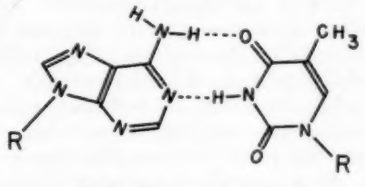

Figure 3. Hydrogen bonding between adenine and thymine in DNA. The R's indicate the linkage to the remainder of each nucleotide chain of the macromolecule.

which has been analyzed contains adenine and thymine in equal amounts and guanine and cytosine (or close relatives of cytosine) in equal quantities, although the proportion of the first pair as compared with the second pair varies over wide limits.

Emphasis should also be placed on the complementary relationship of the two chains of the double helix. Wherever an adenine unit is present in one chain, a thymine must occur in the comparable point in the other chain; where a cytosine is found in one chain, a guanine is found in the other. This relationship provides us with an unique type of molecular unit in DNA.

This, then, is the structure of the gene, a DNA molecule. It is this structure which must provide the answers to the remaining questions concerning the chemistry of gene action.

Chemical Basis for Gene Action

No satisfactory theory existed to explain the precise duplication of the gene, generation after generation, until the complementary nature of the DNA double helix became apparent. This structure leads rather easily to a relatively simple proposal for the mechanism of DNA duplication. This mechanism is indicated in Figure 4. For simplicity a small portion only of a DNA double helix is shown. The first step in the duplication of the original unit may be viewed as its separation into the two constituent polynucleotide chains. The bases of each chain would be free to form H-bonds with the nucleotide units of the metabolic pool. The specificity of the H-bonding and of the steric relationships would insure that the free nucleotides be aligned

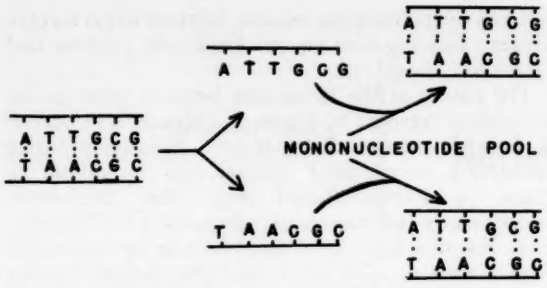

Figure 4. DNA duplication. The straight lines represent the deoxyribose phosphate backbones of DNA chains with the bases, abbreviated as their initial letters, projecting from the chain. The dashed lines are H-bonds.

in a definite, complementary order, a thymine nucleotide being bound to an adenine of one chain, an adenine nucleotide being bound to a thymine of the other, and so on. Enzymatic formation of the phosphodiester linkages would then yield two new double helices, each identical with the original one.

The process is, of course, more complex than this description would indicate. In all probability, for example, the separation of the two chains and the formation of the new ones are intimately related processes occurring in a stepwise manner starting from one or both ends of the unit so that the new double helices are being formed concurrently as the old one separates.

It should be noted that in the new double helices, one chain is an "old" one, one a "new"one. Isotopic tracer studies with living cells confirm that this is indeed the result, providing excellent support for the postulate that each chain of a DNA molecule acts as a form upon which a new specific chain is constructed.

The basis of gene duplication, therefore, appears to be at hand. The action of a gene in bringing about the expression of a characteristic is perhaps less well understood although portions of the process are clear. It is somewhat unfortunate in this regard that most texts on genetics still use as examples of inherited characteristics the obvious, physical ones—eye color, flower color, animal size, and the like. We must realize that a cell does not inherit a characteristic as such but does inherit the potentiality to perform certain chemical reactions, reactions which produce the observed effect. An eye pigment is not inherited; the ability to carry out a number of synthetic reactions leading to the colored compound is inherited. The nature of any cell is determined primarily by the cell's chemical abilities.

In the main a living cell performs only those chemical transformations for which it has available the necessary protein catalysts, the necessary enzymes. It is well established in a number of cases that a gene provides the cell with the information requisite for the synthesis of a single kind of protein molecule, in most cases a certain enzyme. Each gene, therefore, supplies the potentiality for a single kind of chemical reaction by providing the ability to produce the right catalyst for that reaction.

The problem of the synthesis of a certain kind of a protein molecule is essentially that of arranging the monomeric amino acids into the correct positions and then of forming the peptide bonds to link them together in a specific sequence. The gene must, if our ideas are correct, provide a mechanism for the precise ordering of the amino acids in such a peptide chain.

Information that a gene does act in this manner has been gained chiefly from studies of mutant organisms, comparing them with the normal ones. In many cases it has been clearly demonstrated that a mutation involving gene destruction leads to the complete lack of a single enzyme in the mutant organism. Such a case is galactosemia, an inherited inability of humans to metabolize galactose.

A striking example of a more subtle effect on gene structure is provided by another inherited human defect, sickle-cell anemia. Here the gene alteration appears to be slight, probably only the change in sequence of a few nucleotides of a DNA molecule. The effect is that an altered protein molecule is produced, an altered hemoglobin, which differs from the normal hemoglobin only with respect to one amino acid of the chain of about 300 amino acids. The mutation, the gene alteration, results in the synthesis of a protein molecule in which at one point a single incorrect amino acid is incorporated rather than the correct one. The resulting molecule is incapable of normal oxygen transport.

Clearly then, a gene acts by providing the information needed to align amino acids in the right way to yield a specific protein molecule. The chemical mechanisms involved in this process are still largely uncertain although some educated guesses can be made. It appears that in most cases the gene acts in an indirect manner in the process of protein synthesis. Most protein formation occurs in the cytoplasm of cells in small granules called microsomes. The DNA is found only in cell nuclei. This difficulty appears to be reconciled, however, by two lines of evidence. The microsomes are rich in RNA and much circumstantial evidence relates RNA and protein biosynthesis; and RNA is apparently formed in the cell nucleus, migrating then to the cytoplasm. The commonly accepted hypothesis, therefore, is that ribonucleotide units are assembled on a DNA molecule in a specific fashion, utilizing remaining H-bonding possibilities, so that each kind of DNA molecule yields a specific RNA molecule. The RNA molecule in turn, its nucleotide sequence determined by the DNA structure, interacts specifically with the various amino acids to produce a specific amino acid chain, a specific protein.

These considerations give us a reasonable chemical basis for gene action. The nucleic acids are exceedingly complex, very large, relatively unstable, difficult to separate from their close relatives, and beyond our present ability to synthesize *in vitro* with known nucleotide sequences. Much of the evidence, therefore, which has led to the conclusions concerning the nature and mechanisms of action of genes is indirect and difficult to examine within the scope of this paper. However, the results of some of the basic experiments are so important to our understanding of life in chemical terms that a brief glance at their nature seems appropriate.

There is much evidence for the conclusion that a gene is a DNA molecule. The location of DNA in living cells only as part of the chromosomes (known to carry the genetic units), the metabolic stability of

DNA, and the similarity of the action spectrum of UV radiation causing mutation to the absorption spectrum of DNA point to the involvement of DNA as at least part of the genetic material. But the most direct evidence comes from studies of the phenomenon known as bacterial transformation. This is a process which can be viewed as the transfer of a gene from one organism to another by chemical means.

In experiments of this type two closely related organisms are usually used, organisms differing only with respect to the presence or absence of a single gene. A typical example might be two strains of a certain bacterium, one having a gene bringing about the synthesis of an enzyme which inactivates penicillin, the other lacking this gene. The first strain would be penicillin-resistant; the second, penicillin sensitive.

The first step in carrying out transformation with these organisms is to treat cells of the resistant strain in a manner resulting in the rupture of the cell membranes and in the release and solution of the various cellular constituents. The solution is then fractionated chemically to yield the DNA fraction which is extensively purified to remove other substances. The pure DNA is then placed in the medium in which the sensitive strain is growing. After a time, testing with penicillin will reveal the presence of resistant bacteria, bacteria which now have the enzyme catalyzing the destruction of penicillin. Only a relatively small number of the original cells will be transformed, but these are now genetically like the strain from which the DNA was prepared and will continue to multiply in the presence of the antibiotic.

It is difficult to explain such experiments in any way other than to assume that the gene involved in penicillin resistance is a DNA molecule, that it penetrates into a sensitive cell, that it becomes part of the genetic complement of the cell, and that it then promotes the formation of a specific enzyme. The two functions of a gene, self-duplication and formation of a protein, are clearly carried out by material known to be DNA.

This type of experiment has been performed with a number of kinds of microorganisms and with a number of inherited traits. As yet, experiments with higher organisms have not given conclusive results. There is no apparent obstacle to such transformations other than the working out of the precise experimental techniques. The human race is therefore presented with the distinct possibility of being able to control its own heredity. It seems well within our reach to mold any living thing, for good or for evil, into the forms we desire with the abilities we desire, simply by supplying the right DNA molecules. Presumably the first uses of this technique would be the rectification of the diseases caused by genetic deficiencies, diseases which may well become more common in a radioactive world.

Viruses: Nucleic Acids Plus Proteins

There is yet another phenomenon of importance which is closely related to gene action—the virus phenomenon. The viruses are a group of small structures which may have different shapes and sizes but are similar to each other in that they are usually composed of only two kinds of molecules, nucleic acid and protein. The nucleic acid may be either RNA or DNA. The two components are assembled into a structure in which the nucleic acid lies within a protein sheath. The nucleic acid molecules and the protein molecules are held together by H-bonds and by dipole effects, a further example of the organization of macromolecules into specific structures by individually weak interactions. The virus particle is not truly a molecule; neither is it a living organism in the usual sense. By itself it does nothing, but in the proper environment, the interior of a certain kind of living cell, it causes many happenings.

The first step in the course of virus infection is the attachment of a virus particle to a host cell. The nucleic acid then enters the cell, the protein remaining outside. Normal virus infection can indeed be achieved using only preparations of the nucleic acid. The subsequent occurrences in the host cell must be ascribed to the effect of the introduction into the cell of new nucleic acid molecules. The chemical transformations carried out by the cell are rapidly altered away from the normal toward the production of new virus particles, a process requiring the demonstratable formation of new enzymes. Finally, rupture of the cell takes place and the new particles are released, identical in both nucleic acid and protein composition to the original particle. The relation between the effects of gene action, of the transforming DNA, and of virus infection should be apparent. In each case nucleic acid molecules cause their own replication and the formation of protein molecules.

All viruses do not produce immediately harmful results. Some of them repose in cells for long periods without obvious effects, only to be triggered into action by some stimulus. It is difficult in these cases to distinguish between an inherited potentiality and a virus-induced condition. The viral tumors are important examples, for a number of cancerous conditions have been shown to be caused by viruses, sometimes transmitted to the young in the mother's milk. Some workers feel that all tumors are virus-induced, others that mutations of body cells are responsible. Certainly if the nucleic acids of a cell make it what it is, it is logical to assume that cancer cells have abnormal nucleic acids for one cause or another. Much of the chemotherapeutic approach to the cancer problem is based on this assumption, for many of the compounds being used are ones designed to interfere with nucleic acid metabolism.

In this general review of the chemistry of the nucleic acids as expressed in their biological action, I have not intended to minimize the uncertainties and the great amount yet to be learned. Nevertheless, that which we do know about the nucleic acids is of such potential importance to the human race that it would hardly seem out of place to discuss them briefly in a general chemistry course.

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Photosynthesis

Studies leading to a better understanding of photosynthesis have been briefly described in THIS JOURNAL by Kind (*1*) and by Calvin (*2*) within the past three years. I should like to review some recent developments in methods of investigation and to point out areas in which our concept of photosynthesis is changing at the present time.

The process of photosynthesis in plants is often defined by the over-all equation

$$CO_2 + H_2O \xrightarrow[\text{green plant}]{\text{light}} \left\{CH_2O\right\} + O_2$$

which stated in words says that carbon dioxide and water "under the influence of light" are converted by green plants to carbohydrate and oxygen. Such a definition, when used to introduce the subject of photosynthesis to a general chemistry class provides a much too incomplete description of this important process. How might the definition be improved?

First, the essential energy-storing character of the reaction should be emphasized by showing light energy as a reactant in the equation, thus distinguishing photosynthesis from other photochemical reactions in which the role of light is essentially "catalytic." Secondly, in view of present-day knowledge of the direct photosynthesis of products other than carbohydrates it should be stated that the reactants include nitrate (or ammonia), sulfate, and phosphate, and the products include amino acids, fatty acids, phospholipids, and sulfolipids. It will be noted that this statement represents a change from the view held by Kind in 1956 that the immediate reaction of photosynthesis produces carbohydrates only. In addition, a number of other inorganic substances (Fe, Mg, etc.) are required in varying amounts for photosynthesis and may in some cases be incorporated into the products. Finally, if catalysts are to be indicated, then chlorophyll and the many enzymes required should be shown. An equation for the over-all process of photosynthesis might then be given as

$$\text{light} + CO_2 + H_2O + (NO_3^- \text{ or } NH_4^+, HPO_4^=, SO_4^=) \xrightarrow[\text{many enzymes}]{\text{chlorophyll}} O_2 + \left\{\begin{matrix}CH_2O\\ \text{carbo-}\\ \text{hydrate}\end{matrix}\right\} + \left(\begin{matrix}\text{amino acids, lipids,}\\ \text{phospholipids,}\\ \text{sulfolipids, etc.}\end{matrix}\right) \quad (1)$$

Any generalized formula for photosynthesis is a compromise between simplicity and accuracy, but this one at least hints at the complexity of this important process.

For the purpose of discussion, the photosynthetic reaction may be conveniently broken down into three stages. The first of these is the "photo-" part which consists of the absorption of light by the plant pigments and the conversion of the light energy into the stored energy of new chemical bonds of "high energy" compounds (2).

"Photo-": $h\nu$ (light) + low energy compounds $\xrightarrow{\text{chlorophyll}}$ higher energy compounds (2)

An intermediate stage then follows in which the chemical energy of these primary products of the photochemical reaction is utilized in the breaking of the O—H bonds of water and the conversion of two enzymatic co-factors to their more energetic and (for one of them) more reduced forms (3).

higher energy compounds + water + co-factors → O_2 + low energy compounds + high energy co-factors (3)

At the same time molecular oxygen is liberated. We know very little of the detailed mechanism of these reactions, and all or part of this so-called intermediate stage may be in fact a part of the primary photochemical reactions. Indeed, the first stages of photosynthesis may best be considered as the sum of one primary photochemical act in which light energy is absorbed by chlorophyll and a series of non-photochemical steps in which this energy is efficiently transferred through the production of increasingly stable chemical species.

The "-synthesis" stage of photosynthesis is the sum of a great number of enzymatic reactions in which the two co-factors supply the reducing power and energy necessary to bring about the conversion of carbon dioxide (together with nitrate, phosphate, and sulfate) to carbohydrates, amino acids, and lipids (4).

"-synthesis": CO_2 + high energy (and reduced) co-factors ($+NO_3^-$ or NH_4^+, $+ SO_4^=$ etc.) → reduced organic compounds + low energy (and oxidized) co-factors (4)

Let us consider in somewhat more detail these several stages in photosynthesis. We shall not dwell for long on the primary photochemical reactions for the simple reason that although much experimental data has been obtained regarding this process, there is as yet no clearly detailed theory of the chemical mechanism upon which a majority of the investigators in this field can agree. The best that can be said is that in most plants all light energy which is utilized for photosynthesis appears to be employed in one early stage to

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raise the energy content of chlorophyll *a* from its ground state to a higher energy level or "excited state." This is true whether the light energy is absorbed directly by chlorophyll *a* or by other plant pigments, in which case the energy appears to be transferred to chlorophyll *a*.

Then the energy of the excited chlorophyll *a* must somehow be used for the formation of stable compounds with a high content of energy stored in their chemical bonds. There is no unanimity of opinion as to how this is accomplished. One view of the mechanism is illustrated in general terms by the scheme shown in Figure 1. In this scheme the energy of the excited chlorophyll is transferred by some mechanism which

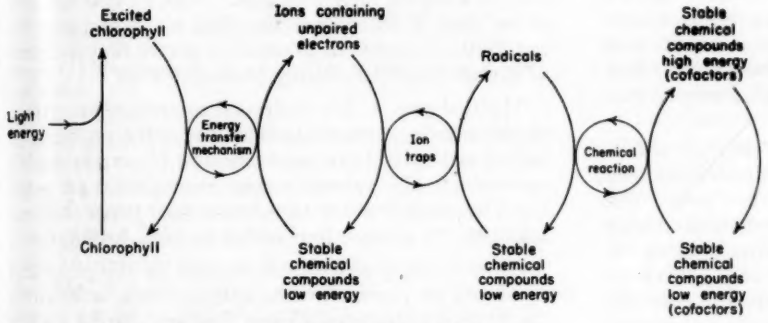

Figure 1. Mechanism of conversion of light energy to chemical energy.

probably involves its migration from one chlorophyll molecule to another through a closely packed sheet of chlorophyll molecules. This sheet of chlorophyll molecules, which is thought to be only one molecule thick, may exist at the interface between lipid and protein layers, the presence of which has been revealed by electron microscope pictures of the chloroplasts. (Chloroplasts are the subcellular units of green cells responsible for photosynthesis.) Eventually (after a millionth of a second or so) the energy packet or "exciton" reaches a point where it is used to supply the energy of ionization of some unknown compound, perhaps chlorophyll itself. For reasons that will be discussed in a moment, this ionization is believed to consist of transfer of electrons in such a way that the resulting ionic species contain "unpaired electrons (*3*)."

Most electrons in organic molecules are found in pairs, so that the spin of one electron, and its resulting interaction with a magnetic field, is cancelled by the equal and opposite spin of its orbital partner. An unpaired electron does have such an interaction, however, and the energy of its interaction with an externally applied magnetic field may change if its spin changes in sign. In order for an unpaired electron to change the sign of its spin in a strong magnetic field in the direction of greater energy it must absorb energy, and this it will do if electromagnetic energy of the appropriate wave length (in the order of 1–5 cm) is supplied. The characteristic energy absorption can be measured by suitable instruments which provide a sensitive means of detection for unpaired electrons. This phenomenon is known as electron spin resonance, or e.s.r.

By means of e.s.r. measurements with thick pastes of live unicellular algae (microscopic green plants) or chloroplasts obtained from spinach, a strong e.s.r. signal has been observed which increases in the light and decreases in the dark. Moreover, a part of this signal is generated even when the plant material is cooled to the temperature of liquid nitrogen (−196°). At this temperature, the movement of atomic nuclei seems improbable, but the movement of an electron, resulting in ionization could easily occur. The detection of light-induced e.s.r. signals at very low temperatures thus forms the basis for the suggestion that one step in the conversion of the energy of excited chlorophyll to the energy of chemical bonds is an ionization process.

At room temperature, the light-induced formation of more than one type of species containing unpaired electrons is indicated by the fact that when the light is turned off, the disappearance of the signal in the dark takes place with more than one characteristic decay time constant. This observation provides evidence for the formation of chemical radicals in which there has been a movement of atomic nuclei. These radicals may form another stage in the transfer of stored energy of chemical bonds.

Finally, stable chemical compounds of high energy content must be formed. In this scheme they would be formed by the interaction of stable chemical compounds of lower energy with chemical radicals. The resulting chemical compounds may be the co-factors required for the reduction of carbon dioxide and nitrate, or they may be other energy-containing compounds which subsequently give rise to the necessary co-factors by chemical or enzymatic reaction. In addition, molecular oxygen is evolved from the products formed by the breaking of the O—H bond of water.

The two energy-carrying co-factors, triphosphopyridine nucleotide (TPNH) and adenosine triphosphate (ATP) are shown in Figure 2. The requirement for these co-enzymes was predicted from studies of the carbon reduction cycle, certain steps of which can only be carried out by the enzymes if the appropriate co-factors are present. TPNH is an electron carrier, transporting electrons obtained by splitting water to the reactions in which they are used for the reduction of carbon dioxide. Each time a molecule of TPNH is oxidized to TPN^+,

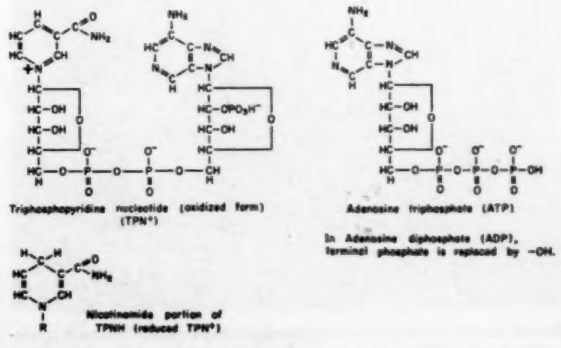
Triphosphopyridine nucleotide (oxidized form) (TPN+)
Adenosine triphosphate (ATP)
In Adenosine diphosphate (ADP), terminal phosphate is replaced by −OH.
Nicotinamide portion of TPNH (reduced TPN+)

Figure 2. Formulas of TPNH and ATP.

two electrons are transferred. The energy stored in ATP is stored through the formation of an anhydride from ADP and inorganic phosphate.

An important stoichiometric relationship between the formation of ATP, TPNH, and O_2 during photosynthesis has been discovered by Arnon and co-workers (*4*, *5*) and is shown in equation 5:

$$\text{light} + H_2O + \text{ADP} + \text{Pi} + \text{TPN}^+ \rightarrow {}^1/_2\,O_2 + \text{TPNH} + H^+ + \text{ATP} \quad (5)$$

This relationship was discovered in studies with isolated chloroplasts from spinach leaves when oxygen evolution, TPNH formation, and ATP formation were measured simultaneously. It will be noted that the reducing co-factor in photosynthesis is TPNH, not diphosphopyridine nucleotide (*1*). This stoichiometric relationship does not tell us what the mechanism of formation of these substances is but does perhaps hint at a pathway that is simpler than we formerly suspected.

We will now turn to the "synthesis" part of photosynthesis and see how these co-factors which have been formed by means of the light reactions are used. The studies of the pathway of carbon reduction during photosynthesis were in a large part carried out in the Lawrence Radiation Laboratory at the University of California by Professor Melvin Calvin and co-workers (*6*). These studies provide an interesting example of the use of radioactive tracer atoms in the elucidation of a complex biochemical pathway. Since carbon dioxide is the sole source of carbon for the photosynthetic reaction, radiocarbon, carbon fourteen, may be introduced very easily into photosynthesizing plants in the form of $C^{14}O_2$ or, for aquatic plants, $HC^{14}O_3^-$ ion.

Let us consider a simple experiment with a suspension of the algae, *Chlorella pyrenoidosa*, which have been very extensively used in these studies. These green, microscopic, unicellular plants, suspended in water containing a few inorganic substances (nitrate, phosphate, etc.), with a stream of $C^{12}O_2$ (ordinary carbon dioxide) photosynthesize at a rapid rate if illuminated from each side in a thin glass vessel. The CO_2 is continually taken up from the solution (where it is in equilibrium with bicarbonate ion) and converted by the photosynthetic plant through a series of biochemical intermediates to various organic products.

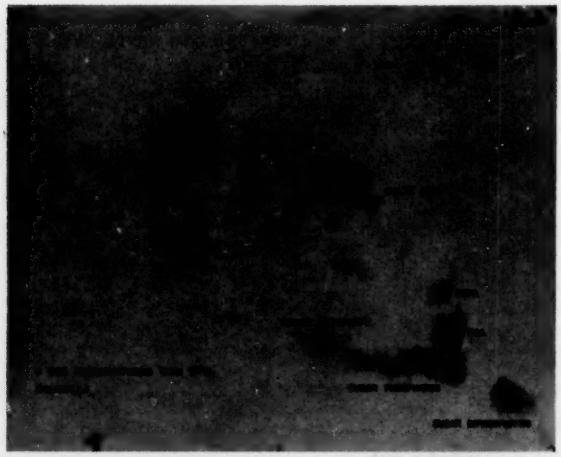

Figure 3. Radioautograph of *Chlorella* extract after 5-second photosynthesis with $C^{14}O_2$.

A solution of radioactive bicarbonate, $HC^{14}O_3^-$, is suddenly introduced into the algae suspension. The plant does not distinguish in any important way between the C^{12} and the C^{14} which are chemically identical, and immediately some of the C^{14} is incorporated into the first of the biochemical products leading from CO_2 to end products. As time passes, the C^{14} would be passed on to subsequent intermediates in the chain. After a few seconds exposure to the C^{14} the suspension of algae is run into methanol to a final concentration of 80% methanol. This treatment denatures all the enzymes instantly and freezes the pattern of intermediates toward further change. Now all that remains to be done is to analyze the dead plant material for radioactive compounds to see what are the first products of carbon reduction during photosynthesis.

The first step in this analysis is to prepare an extract of the soluble compounds since the early products of carbon reduction have been found to be simple soluble molecules. This extract is then concentrated and analyzed by the method of two-dimensional paper chromatography (*7*) as described earlier in THIS JOURNAL (*2*).

Of these many compounds, formed by the plant and separated by paper chromatography, those into which the plant incorporated carbon fourteen during its few seconds of photosynthesis with $HC^{14}O_3^-$ are radioactive and emit the particles resulting from radioactive decay of the C^{14}. In this case these are β^- particles and these may be detected by the fact that they expose X-ray film. Thus, if a sheet of X-ray film is placed in contact with the paper chromatogram, subsequent development of the film will show a black spot on the film corresponding to the exact shape and location of each radioactive compound on the paper. A quantitative determination of the amount of radiocarbon in each compound may then be made by placing a Geiger-Müller tube with a very thin window (to permit the particles to pass through) over the radioactive compound on the paper and counting the emitted particles electronically.

The next stage in the method of radiochromatographic analysis is the identification of the radioactive compounds. This identification is accomplished in a variety of ways including chemical transformation of the compounds which may be eluted from the paper, followed by rechromatography. The final check on the identity of the compound is frequently made by placing on the same spot on filter paper the radioactive compound and 10–100 μg of the pure non-radioactive substance with which the radioactive compound is thought to be identical and chromatographing the two together. A radioautograph is then prepared to locate the radioactive substance after which the paper is sprayed with a chemical spray (for example, ninhydrin for amino acids) which produces a color where the carrier compound is located on the paper. Superposition of the paper chromatogram and the radioautograph (X-ray film) will show an exact coincidence between chemically-developed color on the paper and the black spot on the film if the two substances are identical.

Once the identity of the radioactive compounds formed during a short period of photosynthesis had been established, experiments were performed under a

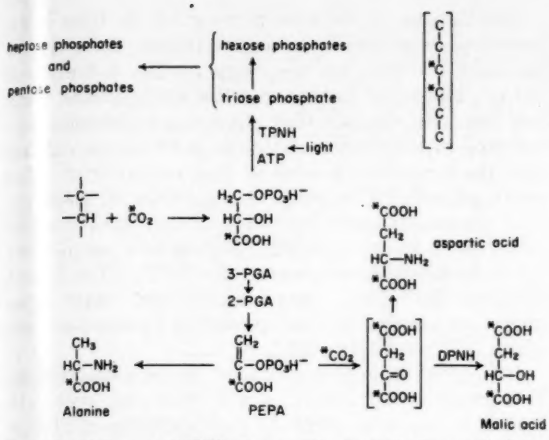

Figure 4. Pathways of $C^{14}O_2$ incorporation in early stages of photosynthesis.

variety of conditions and times of exposure of the algae to radiocarbon. The radioautograph from the experiment with *Chlorella* described above is shown in Figure 3. Even after only five seconds of exposure to C^{14}, a number of compounds are found. Some of these (the sugar phosphates) are not separated from each other by the first chromatography and must be subjected to further analysis. When the sugar monophosphates are hydrolyzed to remove the phosphate groups and rechromatographed, separate spots are found of triose (dihydroxyacetone), tetrose, pentoses (ribulose, xylulose, and ribose), hexoses (glucose and fructose), and heptose (sedoheptulose). The sugar diphosphates are found to include ribulose, fructose, glucose, and sedoheptulose.

After periods of photosynthesis with C^{14} of less than 5 seconds, 3-phosphoglyceric acid (PGA) was found to be the predominant radioactive product. Chemical degradation of this compound showed that the radioactivity first appears in the carboxyl carbon. Later kinetic studies showed that the rate of incorporation of C^{14} into PGA at very short times was much greater than the rate into any other compound. Therefore, it was concluded that PGA is the first stable product of carbon dioxide reduction during photosynthesis, and furthermore, that carbon dioxide first enters the carboxyl group of PGA, presumably via a carboxyllation reaction.

From this point we borrowed from the already known pathways of the glycolytic breakdown of sugars which led to PGA as an intermediate. Noticing that the sugar phosphates are important early products of carbon reduction in photosynthesis, we proposed that they are formed from PGA by a a reversal of the glycolytic pathway. Degradation of the radioactive hexoses from short experiments showed that they were labeled in the two center carbon atoms (numbers 3 and 4) just as one would expect if two molecules of PGA were first reduced to triose and then linked together by the two labeled carbon atoms to give hexose (Fig. 4).

The hexose and triose phosphates may be converted by aldolase or transaldolase and transketolase enzymes to pentose and heptose phosphates. Degradation of these sugars and comparison of the labeling patterns within the molecules showed that this conversion did occur and in such a way that five molecules of triose phosphate were ultimately converted to three molecules of pentose phosphate.

Other known metabolic pathways leading from PGA (Fig. 4) give rise first to phosphoenolpyruvic acid (PEPA) which then may undergo further transformations as follows: (1) it may be carboxyllated and transaminated to give aspartic acid; (2) it may be carboxyllated and reduced to give malic acid; (3) it may be dephosphoryllated and transaminated to give alanine. All of these compounds are labeled after short exposures of the algae to $HC^{14}O_3^-$ in the light. The carboxyllation of PEPA, while a second point of entry for carbon dioxide during photosynthesis, accounts for only 5–10% of the reduced carbon dioxide under normal conditions in *Chlorella*, according to kinetic measurements. Since this reaction converts three additional carbon atoms from PGA to malic acid and aspartic acid at the same time, the amounts of these compounds photosynthesized are significant.

The enzyme system which brings about the oxidation of triose phosphate to PGA in the glycolytic pathway was known to produce ATP and TPNH (or DPNH). If PGA is to be reduced to triose phosphate during photosynthesis, it follows that ATP and TPNH must be supplied. We have already seen that these two co-factors are produced as a consequence of the light reaction and the splitting of water. It might be expected that if the light were turned off from plants photosynthesizing in ordinary carbon dioxide at precisely the same time that $C^{14}O_2$ is introduced, PGA would no longer be reduced to sugar phosphates, but would still be formed (if neither TPNH or ATP are required for the carboxyllation reaction) and would still be used in other reactions not requiring these cofactors. In Figure 5, the radioautograph from just such an experiment, we see that this prediction was correct. Labeled PGA is still formed from $C^{14}O_2$ during 20 seconds in the dark, but only a very little of the PGA is reduced to sugar phosphates. On the other hand, a large amount of alanine is formed from PGA via PEPA in reactions which do not require ATP or TPNH. The small amount of labeled sugar phosphates which does appear is due to the residual ATP and TPNH which was formed while the light was on but which had not yet been used up when the $C^{14}O_2$ was

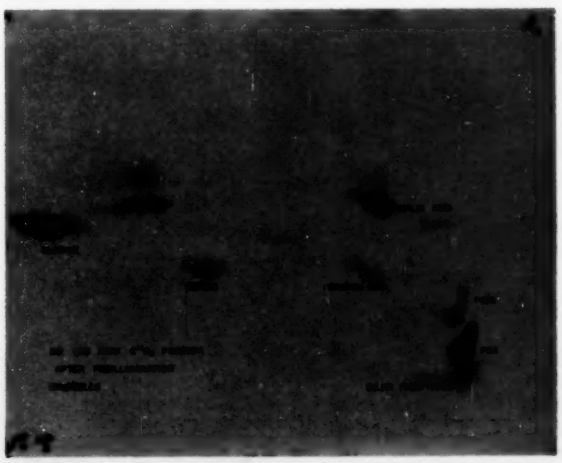

Figure 5. Radioautograph of *Chlorella* extract after 20 seconds with $C^{14}O_2$ in the dark.

introduced. Some malic acid is still formed in the dark, indicating the presence of some DPNH, either remaining from the light or derived from some other metabolic reaction.

Thus far we have not discussed the identity of the source of the compound which undergoes carboxyllation to produce PGA. In order to explain its discovery we must turn to another type of experiment with $C^{14}O_2$ and photosynthesizing algae. In these experiments, algae are first permitted to photosynthesize for 20 minutes or more in the presence of a constant supply of $C^{14}O_2$. During this time all environmental conditions are maintained constant (temperature, CO_2 pressure, light intensity, etc.). After about 10 minutes of exposure to $C^{14}O_2$, so much radiocarbon has passed through the various biochemical intermediate compounds on its way to end products that each carbon atom of each intermediate compound contains, on the average, the same percentage of C^{14} atoms as the CO_2 which is being absorbed. In other words, the specific radioactivities of all the carbon atoms of all the early intermediates are the same as the specific radioactivity of the entering radiocarbon, which can be measured and is therefore known.

At this point, samples of the algae are removed without disturbing the rest of the algae and these samples are killed and subsequently analyzed by the methods which I have already described. The *total radioactivity* of each intermediate is measured, and when this is divided by the known *specific radioactivity*, the total number of carbon atoms of each intermediate compound in the sample can be calculated. Thus the *concentrations* of the various intermediates of the actively photosynthesizing system may be determined.

This determination of the concentrations of intermediates *in vivo* is an extremely valuable tool which has many uses, but let us proceed with the experiment which we had started to describe above. Having taken samples of algae for later determination of the concentrations of intermediate compounds, we now turn off the light and proceed to take a series of samples of the algae as rapidly as we can, which is about every three seconds. When the concentrations of intermediate compounds in these samples are determined, any changes resulting from turning off the light will be revealed. The two most striking changes are found to be in the concentration of PGA which increases rapidly (Fig. 6) and in concentration of one particular sugar, ribulose diphosphate, which drops rapidly to zero.

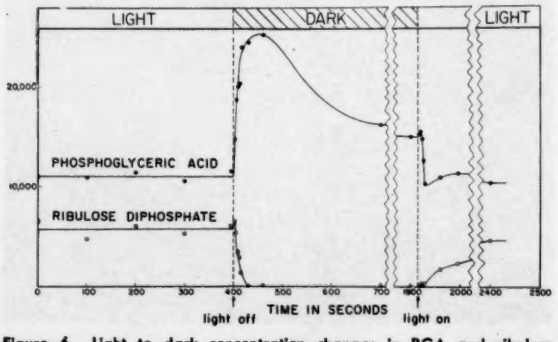

Figure 6. Light to dark concentration changes in PGA and ribulose diphosphate.

The increase in PGA on turning off the light is expected, since we have seen that co-factors, derived from the light reaction, are necessary for the reduction of PGA. The rapid drop in ribulose diphosphate taken together with the fact that other sugar phosphates do not drop rapidly in concentration at first must indicate that the formation of ribulose diphosphate from other sugar phosphates requires a light-formed co-factor. This conclusion agrees with the fact that the known enzyme which converts ribulose-5-phosphate to ribulose-1,5-diphosphate does in fact require ATP. The drop in ribulose diphosphate, alone among the sugar phosphates, means that it is being used up by some reaction which does not require light.

If ribulose diphosphate is used up by some reaction that proceeds in the dark, and if PGA continues to be formed in the dark, could the carboxyllation of ribulose diphosphate to form PGA be the first step in carbon dioxide reduction? To answer this question, another experiment similar to the one just described was performed. This time, however, instead of turning off the light, the light was left on and carbon dioxide was suddenly removed. The result of this experiment (Fig. 7) confirmed the idea of a carboxyllation of ribulose diphosphate, for the concentration of ribulose diphosphate now rose rapidly while PGA dropped rapidly.

The carbon reduction cycle was now complete and is shown in Figure 8. The methods and reasoning leading

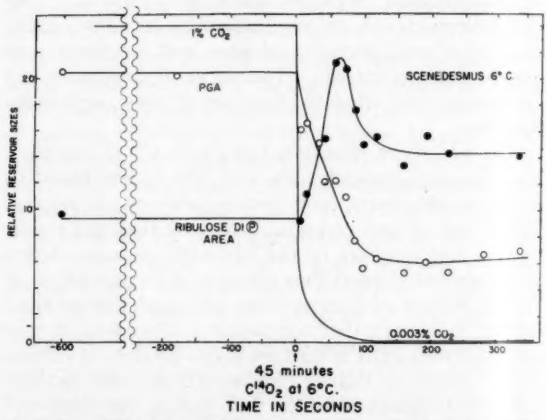

Figure 7. High CO_2 to low CO_2 concentration effect on PGA and ribulose diphosphate concentrations.

to the elucidation of this cycle have been presented more completely elsewhere (*6*, *8*). A complete circuit of the cycle involves the conversion of five molecules of triose phosphate to three molecules of ribulose diphosphate upon which carboxyllation produces six molecules of PGA or its equivalent. There is thus a gain of one three-carbon unit corresponding to the three molecules of carbon dioxide introduced for each turn of the cycle. On the average, five and a fraction of the three-carbon units are reduced to triose phosphate, with five of these going to regenerate ribulose diphosphate while the fraction is employed (presumably) in the synthesis of sucrose, polysaccharides, glycerol, and galactose (constituents of lipids), and other substances. The remainder of the PGA is converted to alanine and serine or, via PEPA, is carboxylated to make four-carbon compounds (Fig. 4).

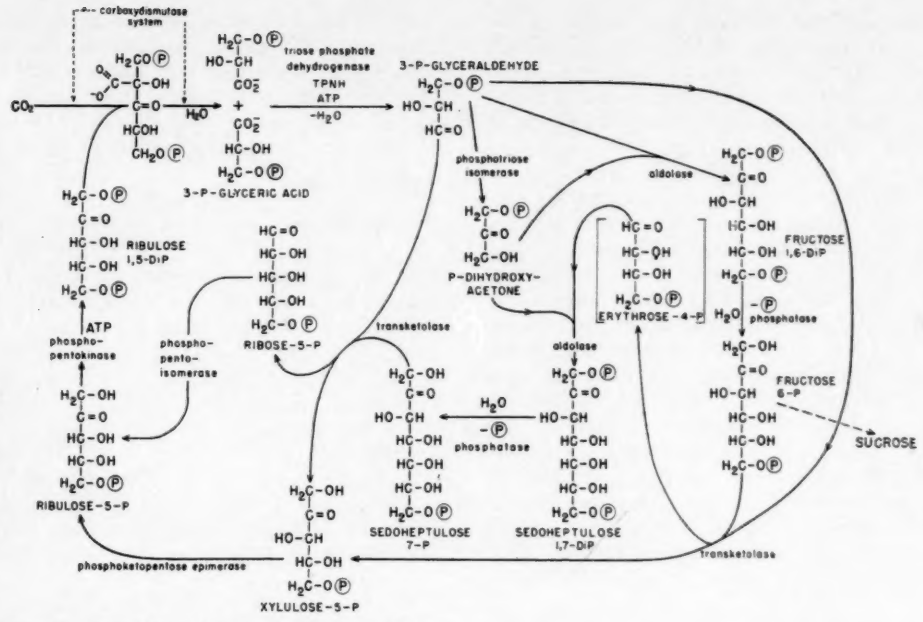

Figure 8. The carbon reduction cycle of photosynthesis.

Rates of Carbon Flow

An interesting problem remaining at this point is the quantitative determination of the relative importance of the various metabolic pathways discussed. The relative rates of the reactions of the carbon reduction cycle are of course fixed, but it is of interest to compare the rates of introduction of carbon dioxide via the two carboxyllation mechanisms with the externally measured uptake of carbon dioxide. Also it is of interest to ascertain the rates of flow of carbon through such secondary intermediates as alanine and aspartic acid and thus gain some idea as to how much amino acids may be synthesized directly from CO_2, via PGA, without the intermediacy of compounds such as sucrose and polysaccharides, once thought to be the sole products of photosynthesis.

The quantitative determination of the flow of carbon via the several pathways is made as follows: First, algae are allowed to photosynthesize with ordinary CO_2 under constant environmental conditions for a few minutes. $C^{14}O_2$ is then introduced, *without any other variation in environmental conditions.* These conditions and the specific radioactivity of the $C^{14}O_2$ are kept constant for the duration of the experiment. Samples of algae are taken rapidly following the introduction of C^{14} and then more slowly until sufficient time has passed to obtain radioactivity saturation of the intermediate compounds. During this time CO_2 uptake is continuously measured by an infrared absorption-measuring instrument which monitors the gas bubbling through the algae in a closed system. C^{14} uptake from the gas is measured by means of an ionization chamber. Thus the specific activity of the $C^{14}O_2$ is continually measured. All of the samples are subsequently analyzed by paper chromatography and radioautography. The C^{14} in each compound in each sample is counted. The growth of radioactivity in each compound from the time of introduction of C^{14} to the time of saturation is thus determined. Typical labeling curves are shown in Figure 9.

As indicated earlier, the concentrations of the intermediates during a period of steady photosynthesis or "steady state" may be calculated from the saturating radioactivity found in the compound and from the specific radioactivity. The *concentrations*, the *growth of radioactivity*, and the *specific radioactivity of the precursor*, all of which are determined in this experiment, may be used in a calculation of the rate of flow of carbon through each intermediate compound. This has been done in one preliminary experiment.

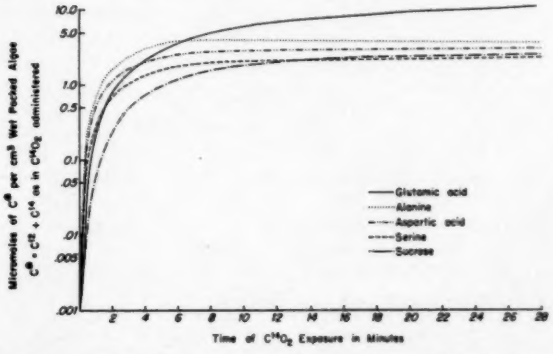

Figure 9. Typical labeling curves of biochemical intermediates in photosynthesis.

Among the conclusions derived from the calculation of rates of carbon flow through various intermediates and along certain pathways are the following: (1) at least 85% of the assimilated carbon dioxide, as measured externally, is found to enter the reduction pathways via the carboxyl group of PGA. The carbon reduction cycle is therefore by far the most important pathway for the incorporation of carbon dioxide during photosynthesis. (2) Five per cent or more of the entering carbon dioxide enters via carboxyllation of PEPA leading to malic acid and aspartic acids and probably to

glutamic acid, although the pathway to the latter compound is not completely understood. Altogether, about 20% of all the carbon taken up finds its way into amino acids in photosynthetic reactions under the conditions of this experiment. These amino acids (including alanine and serine) presumably give rise to other amino acids and eventually to protein.

In conclusion, it may be said that through the employment of tracer elements, particularly carbon fourteen, the pathway of carbon reduction during photosynthesis has been mapped and the resulting pathway has been demonstrated quantitatively to account for most or all of the carbon reduced during photosynthesis. From the nature of this pathway, the requirements for energetic co-factors, derived ultimately from the light reaction, have been established. The detailed mechanism by which these co-factors are formed in the light reaction, as well as the mechanism by which water is split and O_2 is evolved, are not as yet known, but studies with isolated chloroplasts have demonstrated a stoichiometric relation between the evolution of oxygen and the formation of both co-factors, ATP and TPNH. Electron spin resonance measurements of plants in the light suggest the possible formation of ions containing unpaired electrons and free radicals in the early stages of the conversion of light energy to the stored energy of chemical bonds.

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Photochlorination of Hydrocarbons with Chlorine Generated *in Situ*

A Student Laboratory Exercise

Fair yields of chlorocyclohexane have been obtained simply and safely by irradiating a heterogeneous mixture of cyclohexane (1 mole), concentrated hydrochloric acid (100 ml), and manganese dioxide (Baker and Adamson, 95% "precipitated," 0.2 mole). Chlorine generated in a lower aqueous layer by the well-known reaction

$$MnO_2 + 4HCl \rightarrow MnCl_2 + Cl_2 + 2H_2O$$

rises into the supernatant hydrocarbon layer where, under the influence of light from a 300-watt incandescent lamp, the substitution reaction takes place:

$$Cl_2 + C_6H_{12} \rightarrow C_6H_{11}Cl + HCl$$

In adapting the experiment to performance by each individual of a large laboratory group beginning their study of organic chemistry, a simple apparatus was constructed that permitted irradiation of six reaction flasks at once. Irradiation time was 8–10 hours, and thirty flasks could be processed in a week. During one laboratory period, each student places the cyclohexane and manganese dioxide in a 500-ml Florence flask fitted with an air-cooled condenser, and leaves it with the instructor. Addition of hydrochloric acid and irradiation are carried out between laboratory periods by the instructor or supervisor of laboratory work. The next laboratory period is devoted to working up the reaction product.[1]

Besides demonstrating a photochemical process and an important reaction of hydrocarbons which has preparative value, the exercise provides early practice in typical laboratory techniques and calculations. The choice of cyclohexane because it can give only one monochlorination product is worth pointing out to the students as is the use of an excess of cyclohexane to limit polysubstitution.

In a preliminary investigation of the scope of the chlorination method described, toluene gave benzyl chloride, identified by conversion via the Grignard reagent and phenyl isocyanate to phenylacetanilide. Benzene gave the *addition* product, hexachlorocyclohexane. Two of the geometrical isomers were isolated by standard procedures:[2] the *alpha* isomer, mp 155°, and the *beta* isomer, mp 295–300°. From diphenylmethane, we obtained practical yields of benzhydryl chloride, identified by its hydrolysis to benzhydrol, mp 64–65°, and dichlorodiphenylmethane, identified by its conversion to benzophenone 2,4-dinitrophenylhydrazone.

[1] A detailed description of apparatus and procedure will be furnished upon request.
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Chelon Approach to Analysis (I)
Survey of Theory and Application

The introduction of chelons[1] such as EDTA has virtually revolutionized the analytical approach to metal ion analysis. This approach commenced with the classical work of Schwarzenbach (*31–33*) on the volumetric determination of calcium and magnesium—a procedure which was rapidly and universally adopted for estimation of permanent water hardness. Since then the applicability of the EDTA titration has been extended to the determination of over 50 elements (*3, 5, 30, 39*), and its scope will undoubtedly be broadened even further.

In Table 1, the elements enclosed in solid lines have been estimated by direct or back titrations with EDTA, and the elements (or their compounds) enclosed in dashed lines indirectly, i.e., via another element after precipitation. For example, sulfur in sulfate is determined indirectly through precipitation with excess barium ion and back titration of the unreacted barium ion with EDTA. Substances which may be reduced with the aid of liquid amalgams (such as reducible organic compounds) will, under suitable conditions, liberate an equivalent quantity of metal ion (M^{+n}):

$$Ox^{m+n} + M(Hg) \rightarrow Red^{+m} + Hg + M^{+n} \qquad (1)$$

which can then be titrated chelometrically. Elements such as silver ion, which by exchange reactions liberate directly titratable elements

$$2Ag^{+} + Ni(CN)_4^{-} \rightarrow 2Ag(CN)_2^{-} + Ni^{++} \qquad (2)$$

fall into this category. The remaining elements in Table 1 have not *yet* been determined by a chelometric titration.

Because of the present wide applicability of the chelon approach and because of its unusual future possibilities, this brief and introductory status report is given to acquaint chemists with this approach and to present some material suitable for use in analytical courses. A demonstration lecture on EDTA and complex formation by Johnston, Barnard, and Flaschka (*9*) serves as a suitable qualitative introduction to the quantitative approach presented here.

Table 1. Elements Determinable by EDTA Titrations

Li	Be											B	C	N	O	F
Na	Mg											Al	Si	P	S	Cl
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I
Cs	Ba	*	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At
Fr	Ra	**														
*Lanthanides		La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
**Actinides		Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	103

Based in part on work supported by Air Force Office of Scientific Research, Air Research and Development Command, under contracts AF18(600)-1160 and AF49(638)-333.

[1] A generic term for a class of reagents including polyaminocarboxylic acids, polyamines, and related compounds which form stable, soluble, usually 1:1 complexes with metal ions and may consequently be employed effectively as titrants for metal ions.

The Design of Chelons (*4, 12, 13, 28, 29*)

The classical titration of acid with strong base is well known

$$H^{+} + OH^{-} \rightarrow H_2O \qquad \log K = 14 \qquad (3)$$

It is obviously a practical analytical titration in view of the large value of log K. Similarly the titration of acid with weaker bases such as ammonia or cyanide

ion is also applicable. G. N. Lewis (*11*) considered complex formation as a type of acid-base reaction. Thus the reaction

$$Cu^{++} + 4NH_3 \rightarrow Cu(NH_3)_4^{++} \qquad \log K = 12.6 \qquad (4)$$

might constitute a suitable means for titrating Cu^{++} ions, copper in this case being the acid (electrophilic) and ammonia the base (nucleophilic). Similarly one might consider the titration of cadmium with cyanide ion, according to

$$Cd^{++} + 4CN^- \rightarrow Cd(CN)_4^{--} \qquad \log K = 18.8 \qquad (5)$$

In view of the large equilibrium constants for these complex formation reactions, such titrations might appear feasible since they are somewhat analogous to the titration of acid with base. However, the resulting titration curves (*13*) illustrate that, although the titration of hydrogen ions with base yields adequately sharp end points, the titration of metal ions with a simple ligand (such as ammonia or cyanide) usually yields drawn out and completely unusable end point breaks.

This disappointment arises *not* from the impressive values of log K for the reactions written above, but rather from the stepwise formation of the metal complexes. Consider the titration of copper with ammonia proceeding by two *extreme* paths. After 4 ammonia molecules have been introduced, the solution may contain:

Path 1
$Cu(NH_3)^{++}$ $Cu(NH_3)^{++}$ $Cu(NH_3)^{++}$ $Cu(NH_3)^{++}$ Cu^{++} . . .
Path 2 $Cu(NH_3)_4^{++}$ Cu^{++} Cu^{++} Cu^{++} . . .

Had path 2 been followed, a suitable sharp end point would have been obtained in the titration of fairly concentrated solutions and such methods would already have found wide use. However, the prevalent reaction initially is more nearly path 1. This mode of complexation is attributed to the fact that the $Cu(NH_3)^{++}$ is effectively less acidic than the "free" (hydrated) copper ions, Cu^{++}. Thus, as ammonia is added to a solution containing Cu^{++} as well as $Cu(NH_3)^{++}$, the ammonia reacts in preference with the free Cu^{++} ions.

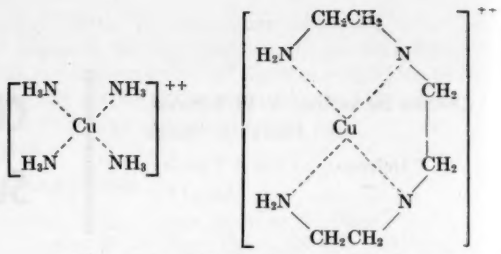
The relative Lewis acidities of the hydrated copper ions and the various copper ammines is shown by the values for the formation constants

$$\begin{aligned}
Cu^{++} + NH_3 &\rightleftarrows Cu(NH_3)^{++} && \log K_1 = 4.1\\
Cu(NH_3)^{++} + NH_3 &\rightleftarrows Cu(NH_3)_2^{++} && \log K_2 = 3.5\\
Cu(NH_3)_2^{++} + NH_3 &\rightleftarrows Cu(NH_3)_3^{++} && \log K_3 = 2.9\\
Cu(NH_3)_3^{++} + NH_3 &\rightleftarrows Cu(NH_3)_4^{++} && \log K_4 = 2.1 \qquad (6)
\end{aligned}$$

The stepwise formation of the copper ammine complexes causes the free copper ion concentration to decrease more rapidly during the course of the titration than it would have by path 2. Consequently the value of pCu ($-\log [Cu^{++}]$) increases rather rapidly upon the addition of ammonia and no strong inflection is obtained at the equivalence point.

Even had path 2 been followed, the binding of copper to four ammonia molecules would decrease rapidly upon dilution and thus dilute solutions of copper could not be titrated. This results from the fourth power dependence on ammonia in the equilibrium constant expression.

Both these effects can be overcome by the chelon approach. Compare complexes (I) and (II).



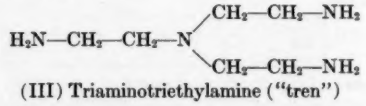
(I) Ammonia (II) Triethylenetetramine ("trien")

In order to get this exclusive formation of the copper-tetrammine and thus a reaction by path 2 rather than path 1, the following imaginary approach might be considered. If copper were added to a very concentrated solution of ammonia, the copper tetrammine would be the prevalent form of complex ion in solution. Hence if such a solution were packaged in an ammonia-impermeable membrane such that each package contains four ammonia molecules, then these little packages of concentrated ammonia could be added to a copper ion solution and a very effective titration obtained. An easier and certainly a more practical approach to packaging the nitrogen ligands into a small volume is to join each of the four basic nitrogens by an ethylene linkage as shown in (II). In this way triethylenetetramine may be considered as an extremely small droplet of a concentrated ammonia solution.

Furthermore, the chelon titrant is now a single molecule and, because the reaction is 1:1 (one copper to one triethylenetetramine), the dissociation of the resulting complex does not increase so rapidly upon dilution. It is actually the 1:1 complex and the preconcentration of the ligand atoms, rather than the formation of "five-membered rings" in the resulting complex, which give rise to the well-known *chelate effect*.

The nitrogens could have been connected by linkages such as $-CH_2CH_2CH_2-$ but the nitrogens would then be even further removed from one another and the "droplet" would be less concentrated in basic nitrogen. This is less desirable since weaker chelates would be formed. Ring strain in the resulting structure also needs consideration.

The nitrogens might be joined by ethylene linkages in a different way as shown in (III)



(III) Triaminotriethylamine ("tren")

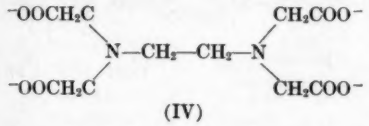
The nitrogens in (III) are concentrated approximately as effectively as in triethylenetetramine. However, bonding of (III) with metal ions of square planar configuration (e.g., Cu^{++}) is not nearly so effective as that of the linear triethylenetetramine, (II). This is attributed to the fact that *all* the nitrogens in (III) cannot be used for coordination with copper without a considerable strain in the resulting complex.

On the other hand, the spider-like configuration of (III) is well suited for complexation of metal ions which bond in a tetrahedral mode, such as zinc. This

structural factor is borne out by the following stability constants (*1*).

$$\begin{aligned} Cu^{++} + \text{trien} &\rightleftarrows Cu(\text{trien})^{++} && \log K = 20.4 \\ Cu^{++} + \text{tren} &\rightleftarrows Cu(\text{tren})^{++} && \log K = 18.8 \\ Zn^{++} + \text{trien} &\rightleftarrows Zn(\text{trien})^{++} && \log K = 12.1 \\ Zn^{++} + \text{tren} &\rightleftarrows Zn(\text{tren})^{++} && \log K = 14.7 \end{aligned} \quad (7)$$

Another feature to be considered in the design of chelons is that certain metal ions (such as Co, Ni, Cu, Zn, Cd, and Hg) form more stable coordination bonds with basic nitrogen than with oxygen (*34*); in ammoniacal solution such metal ions form stable ammine complexes. In contrast, other metal ions (such as alkaline and rare earths, Al, Bi, Pb, and Sc) either do not react or form hydrous oxide precipitates. Thus the all-nitrogen type chelons, such as (II) or (III), complex with a rather restricted group of metal ions. By incorporating *both* oxygen and nitrogen atoms as ligands in a chelon, a more general complexing reactivity is accomplished. The structure of *e*thylene*d*iamine*e*tetra*a*cetate (abbreviated EDTA)



(IV)

contains two nitrogen (ammonia-type) ligands and four oxygen (acetate-type) ligands and each molecule behaves as though it were a highly concentrated droplet of ammonia and acetate ions—*but forms a 1:1 complex!* Figure 1 illustrates its *general* complexing ability in titrations.

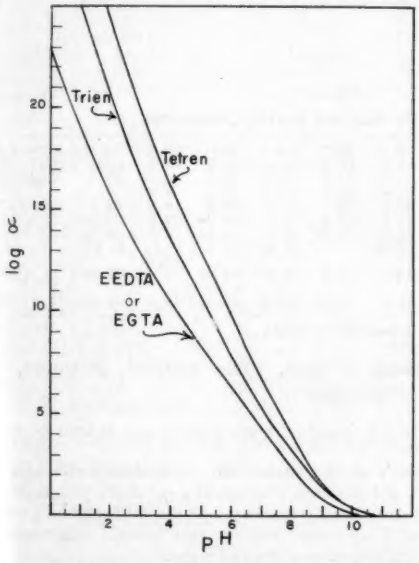


Figure 1. The dependence of log α on *p*H for Trien, Tetren, EEDTA, and EGTA.

It is easily seen that EDTA represents a structure containing about the highest obtainable pre-concentration of nitrogen and oxygen ligands and thus is almost ideal for general complexing of metal ions. Homologous reagents containing 1, 3-diaminopropane and propionate arms (in various combinations) have been synthesized and studied. In these cases less reactivity has been found in accord with the expected results.

Various steric effects have also been accomplished by modifying the EDTA molecule through substituting methyl group(s) on the ethylene linkage and by replacing ethylenediamine with cyclized compounds such as 1,2-cyclohexane diamine. Occasionally somewhat greater stability was found and may be attributed to a slight preorientation of the ligands and increased basicity of the nitrogen ligands. Diethylenetriaminepentaacetic acid (DTPA) is closely related to EDTA in structure but has more ligand groups and forms definitely more stable complexes. Other possibilities consist of changing the nitrogen ligands for phosphorus and the acetate ligands for hydroxyethyl, thiol, or phosphonate groups. The greater success has arisen from the introduction of a hydroxyethyl group in place of one of the acetate groups. The resulting compound forms an exceedingly strong complex with iron(III) although its complexing tendency with many other metal ions is weaker.

Available Chelons and Their Properties

A great number of chelons have been synthesized and a few of them are now commercially available. Tables 2 and 3 summarize the properties and sources of some of these.

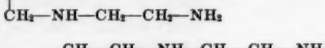
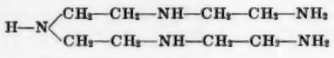
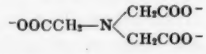
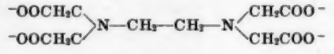
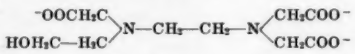
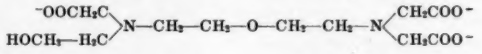
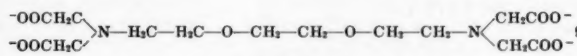
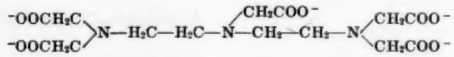
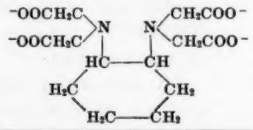
By comparing the different stability constants for calcium and magnesium, we find that EGTA forms a much more stable complex with calcium ($\log K = 11.0$) than with magnesium ($\log K = 5.4$). This difference permits the selective titration of calcium in the presence of magnesium (*26*, *27*) at *p*H 9, whereas, with EDTA, calcium ($\log K = 10.7$) and magnesium ($\log K = 8.7$) are cotitrated at this *p*H value.

The all-nitrogen type chelons, such as triethylenetetramine (trien) and tetraethylenepentamine (tetren), form stable complexes with a fairly restricted set of metal ions and thus may be used for the selective titrations of these ions in the presence of the others (*15*, *16*, *21*). Also copper can be readily titrated in the presence of nickel, zinc, and cadmium.

As discussed more fully later, extreme care must be taken when comparing the values of the *absolute* stability constants for a given metal with various chelons. From Table 3, copper would seem to form more stable complexes with trien than with EDTA; while this is true at high *p*H, the reverse is true at low *p*H due to the "*p*H effect." In contrast, comparison of the ratio of the absolute stability constants for a given chelon with two metal ions is generally in agreement with their effective stability. For most accurate comparisons, account must be taken of the formation of metal chelonate derivatives ("mixed" complexes) such as MHY^{-}, $M(OH)Y^{-3}$, $MY(NH_3)^{-2}$, MY (halides) because their formation will increase the net stability of the metal complex.

Not to be overlooked in the choice of the chelon is the purity of the commercially available material. For example, the technical grades of trien and tetren are most unsuited for the titration purposes because of contamination with lower amines. These lower amines react with metal ions only slightly less efficiently than the present chelon and cause the end points to be drawn out and barely detectable. Hence old solutions

Table 2. Chelons Most Frequently Used in Metal Titrations

Chelon	Abbrev.	Formula	Proton formation (acidity) constants (log) H_1	H_2	H_3	H_4	H_5
Triethylenetetramine *a, c, h*	Trien		9.92	9.20	6.67	3.32	..
Tetraethylenepentamine *h*	Tetren		10.0	9.2	8.2	4.1	2.6
Nitrilotriacetic Acid *a–d, f* Ammoniatriacetic Acid	NTA		9.73	2.49	1.9	...	...
Ethylenediaminetetraacetic acid *a–g*	EDTA		10.26	6.16	2.76	2.0	...
N-Hydroxyethylenediaminetriacetic acid *d, f*	HEDTA		9.73	5.33	2.64	...	...
Ethyletherdiaminetetraacetic acid *d, f*	EEDTA		9.49	8.82	2.67	1.90	...
Ethylene glycol-bis-(β-aminoethyl ether)-N,N′-tetraacetic acid *d, f*	EGTA		9.43	8.85	2.68	2.0	...
Diethylenetriamine-pentaacetic acid *d, f*	DTPA		10.55	8.60	4.27	2.41	2.08
Cyclohexanediaminetetraacetic acid *b, d, f, g*	CyDTA		11.70	6.12	3.52	2.40	...

a J. T. Baker Chemical Co., Phillipsburg, N. J.
b Chemapol Chemical Co., Panská 9, Prague 3, Czechoslovakia.
c Distillation Products Industries, Rochester 3, N. Y., (technical grade polyamines). LaMont Laboratories, 5002 West Mockingbird Lane, Dallas 9, Texas, supplies NTA, EDTA, HEDTA, DTPA, CyDTA.
d Dojindo and Co., Ltd., 718 Izumimachi-Ima, Kumamoto-Shi, Japan.
e Fisher Scientific Co., Pittsburgh, Pa.
f Geigy Industrial Chemicals, Saw Mill River Road, Ardsley, N. Y.
g Hach Chemical Co., Ames, Iowa.
h Union Carbide Chemical Co., Charleston, West Virginia (technical grade polyamines only).

Table 3. Stability Constants of the Most Frequently Applied Metal Chelonates

Chelon	Mg^{+2}	Ca^{+2}	Sr^{+2}	Ba^{+2}	La^{+3}	R.E.$^{+3}$	Mn^{+2}	Fe^{+2}	Fe^{+3}	Co^{+2}	Ni^{+2}	Cu^{+2}	Zn^{+2}	Cd^{+2}	Hg^{+2}	Al^{+3}	Pb^{+2}
Trien	Negl	Negl	Negl	Negl	Negl	...	4.9	7.8	...	11.0	14.0	20.1	11.9	10.8	25.0	Negl	10.4
Tetren	Negl	Negl	Negl	Negl	Negl	...	7.0	...	...	15.1	17.8	22.9	15.4	14.0	27.7	Negl	10–11
NTA	5.4	6.4	5.00	4.8	10.4	10.4–12.2	7.4	8.8	15.8	10.4	11.5	12.6	10.5	9.8	...	...	11.1
EDTA	8.7	10.7	8.7	7.9	15.5	15.8–19.8	13.8	14.4	25.1	16.3	18.6	18.8	16.5	16.5	22.1	16.1	17.9
HEDTA	7.0*a*	8.0	6.8*a*	6.2*a*	13.2	14.1–15.8	10.7	11.6	...	14.4	17.0	17.4	14.5*a*	13.0	20.1*a*	...	15.5*a*
EEDTA	8.3	10.0	8.6*a*	8.2	...	...	13.2	...	...	14.7*a*	14.7*a*	17.8	15.3*a*	16.3	23.1*a*	...	14.4*a*
EGTA	5.4	10.9*a*	8.5	8.4	...	...	12.3*a*	...	...	12.3*a*	13.6*a*	17.8*a*	13.0	16.7*a* 14.5*a*	23.8*a*	...	14.6*a*
DTPA	9.0	10.7*a*	9.7	8.6	...	19.1*a* (La)	15.5*a*	16.7	27.5	19.0	20.2	21.0	18.8*a*	19.0*a*	27.0*a*	...	18.6*a*
CyDTA	10.3	12.3*a*	10.0*a*	8.0	...	16.8–21.5	16.8	...	...	18.9	19.4*a*	21.3	18.6*a*	19.2	24.4*a*	17.6	19.7

a Unpublished data by Holloway, J. H., and Reilley, C. N., obtained potentiometrically with mercury electrode.

of such polyamines may yield less well defined end points than fresh solutions despite the fact that their titer may have changed only slightly. Although limited information is now available, the chelons listed in Table 3 are reasonably stable in aqueous solution and each may serve effectively as a titrant.

End Point Methods

Titration of Liberated Acid

This method constitutes the original end point technique and is based upon estimating the acid liberated upon complex formation

$$M^{++} + H_nY^{n-m} \rightleftarrows MY^{2-m} + nH^+$$

In this approach, the sample solution of metal ion is first neutralized with base and then an excess preneutralized solution of chelon is added. The hydrogen ions liberated by the above reaction are now titrated with standard base. The method, however, suffers several difficulties.

Many metal ions hydrolyze readily and cannot be truly preneutralized.

The pK_a's of the chelon are often closely spaced and thus indistinct end points are obtained even if the pre-neutralization point is taken exactly midway between adjacent pK_a values.

The metal ion sample solution may contain constituents which buffer in the *p*H range of the end point.

The metal chelate itself may form complexes of the type H_nMY or $MYOH_n$ in the *p*H range of the end point.

Because of these disadvantages, this end point procedure is seldom employed except in theoretical studies.

Metallochromic Indicators

This approach is by far the most popular at the present time and has served to focus considerable favorable attention on the practicality of the chelon approach to the titration of metal ions. It consists of

Table 4

Ion	Method	Conditions, *p*H	End point color change
Conditions for Some Recommended Visual EDTA Titrations			
Al	D; Cu-PAN	Ac; 3Δ	Re-Ye
	B (Mn-Zn); Erio T	NH_3; 10, Asc.	Bl-Re
Ba & Sr	D; MTB	$(ET)_2NH$; 11.5–12.7	Bl-Cls.
	S; (MgY); Erio T	NH_3; 10	Re-Bl
Bi	D; X. Or.	HNO_3; 0.1–0.2 *M*	Re-Ye
	D; PVlt	HNO_3; 2.5	Bl-Ye
Cd	D; Erio T	NH_3; 10	Re-Bl
	D; Cu-PAN	Ac; 4.5Δ	Vlt-Ye
Ca	D; Calcon	NaOH or $(Et)_2$-NH; 12.5	Re-Bl
	D; P and R dye	NaOH; 12–14	Pi-Bl
	B; (Mg) Erio T	NH_3; 10	Bl-Re
	S; (MgY) Erio T	NH_3; 10	Bl-Re
Cr(III)	B; (Mn, Zn); Erio T	NH_3; 9–10, Asc.	Bl-Re
Co	D; Cu-PAN	Ac; 3Δ	Re-Ye
	B; (Mn, Zn); Erio T	NH_3; 9–10, Asc.	Bl-Vlt
Cu	D; PAN	Ac; 3,4.5Δ	Vlt-Ye
Ga	D; Cu-PAN	Ac; 3–3.5Δ	Re-Ye
	B; (Pb-Zn); Erio T	NH_3; 9.5, tar.	Bl-Re
In	D; Erio T	NH_3, tar., Δ	Re-Bl
	D; PAN or Cu-PAN	Ac; 2.5–3.5Δ	Re-Ye
	B; (Mg, Zn); Erio T	Ethylene-diamine; 10	Bl-Re
Fe	D; Cu-PAN	Ac; 4.5Δ	Vlt-Ye
	D; sal., sulfsal., Tir.	Ac; 2–3	Br; Re; Bl *to* Ye
Pb	D; Erio T	tar; NH_3, 10	Vlt-Bl.
	D; Cu-PAN	Ac; 4.6Δ	Re-Ye
	D; X. Or.	Ac; 5–6	Re-Ye
	B (Mg, Zn); Erio T	NH_3; 10	Bl-Re
Mg	D; Erio T	NH_3; 10	Re-Bl
Mn	D; Erio T	NH_3; 10, Asc. or NH_2OH	Re-Bl
	B (Mg, Zn); Erio T	NH_3; 10, Asc.	Bl-Re
Hg(II)	D; Cu-PAN	Ac; 3–3.5Δ	Vlt-Ye
	B (Mg, Zn); Erio T	NH_3; 10	Bl-Re
Ni	D; Cu-PAN	Ac; 3Δ	Re-Ye
	B (Mn, Zn); Erio T	NH_3; 10, Asc.	Bl-Vit
Pd	B (Zn); Erio T	NH_3; 9–10	Bl-Re
Sc	D; Erio T	NH_3; malic acid, 9–10Δ	Re-Bl
	B (Cu); PAN	Ac; 2.5Δ	Ye-Re. Vlt
Tl(III)	S $(MgY)^{-2}$; Erio T	NH_3; 10	Re-Bl
Th	D; PVlt	HNO_3; 40°, 2.5–3.5	Re-Ye
	D; X. Or.	HNO_3; 2.5–3.5	Re-Ye
	B (Bi); PVlt	HNO_3; 2.5–3.5	Ye-Bl
V(IV)	D; Cu-PAN	Ac; 3.5, Δ	Re-Ye
	S $(MgY)^{-2}$, Erio T	NH_3; 10	Re-Bl
Y & R.E.'s	D; Erio T	NH_3; tar. or cit., 8–9, Δ	Re-Bl
	B (Zn); Erio T	NH_3; 8–9	Bl-Re
Zn	D; Erio T	NH_3; 10	Re-Bl
	D; Cu-PAN	Ac; 4–5, Δ	Vlt-Ye
	D; Znc	NH_3; 9–10	Bl-Te
Zr (Hf)	D; X. Or.	HNO_3 (1*M*); Δ	Re-Ye
	B (Fe^{+3}); sal. acid	NH_4Ac; 5–6	Cls-Br
Conditions for Some Indirect Visual EDTA Titrations			
N (as NH_3)	Add excess HgY^{-2}; titr./w. NaOH, Bromthymol blue and red screening dye	*p*H 6.8	Ye-Grey
S (as SO_4^{--})	Precipitate as $BaSO_4$ titr. excess Ba^{++}w. EDTA	See Ba^{++}	
P (as PO_3^{--})	Precipitate as $MgNH_4PO_4 \cdot 6H_2O$; titr. precipitate for Mg^{++}, Erio T	NH_3; 10	Re-Bl

Additional information on many of the mentioned procedures and references will be found in the Baker brochure (*3*) and references *5*, *30*, and *37*.

Abbreviations: D, direct titration; B, back titration; S, substitution titration; Erio T, Eriochrome Black T[a–e]; Calcon[a, e]; PAN, 1-(2-Pyridylazo)-2-naphthol[a,c,d]; PVlt, Pyrocatechol Violet[a–e]; MTB, Methylthymol Blue[b]; P and R dye, Patton and Reeder indicator[c,d]; sal., salicylic acid; sulfasal, sulfosalicylic acid[a]; Tir., Tiron[a–d]; X. Or., Xylenol Orange[b–d]; Zcn, Zincon[a,c,d]; Bl, blue; Vlt, violet; Pi, pink; Br, brown; Cls, colorless or gray; Re, red; Ye, yellow; Ac, acetate buffer; NH_3, ammonia buffer; Asc. ascorbic acid; tar, tartrate; cit, citrate; Δ, heat to 90°C.

[a] J. T. Baker Chemical Co., Phillipsburg, N. J.

[b] Chemapol Chemical Co., Panská 9, Prague 3, Czechoslovakia.

[c] Dojindo and Co. Ltd., 718 Izumimachi-Ima, Kumamoto-shi, Japan.

[d] Fisher Scientific Co., Pittsburgh, Pa.

[e] Hach Chemical Co., Ames, Iowa.

LaMont Laboratories, 5002 Mockingbird Lane, Dallas 9, Texas, supplies a number of these indicators.

adding a trace quantity of a complexing dye, called a metal indicator or a "metallochromic indicator," which exhibits one color in the "metallized" form and different color in the "unmetallized" form.

$$M^{+z} + HIn^{m-1} \rightarrow MIn^{m+1} + H^+$$
(e.g., blue) (e.g., red)

Thus during the major part of a direct titration, the metallochromic indicator exists entirely in the metallized form, only the free metal ion remaining in solution being titrated with the chelon. At the equivalence point, the chelon removes the metal ion from the metal-dye complex with a resulting color change. Sharper end points are obtained when extremely small concentrations of metallochromic indicators are employed. Metallochromic indicators should, therefore, have large molar extinction coefficients in the visible region making possible their use in extremely dilute concentrations (approximately 10^{-5} to $10^{-6}M$). The metallochromic indicator is itself a multidentate chelating agent containing, for example, polyaminocarboxylate, hydroxy, basic nitrogen, and/or azo ligands. One of the protonated ligands is potentially conjugated to the ring and hence chelation with this group removes the proton, alters the resonance properties of the dye, and causes a color change.

For a metallochromic indicator to be useful, a proper sequence of effective stability constants must be met: the effective stability of the metal-chelon must be greater than that of the metal-indicator and yet the metal indicator must be sufficiently stable to exist as metallized complex in extremely dilute solutions. Few substances allow this necessary sequence. Hence a given metallochromic indicator may be useful for a few metals under highly restricted *p*H and buffer (type and concentration) conditions, and yet may be entirely unsuitable for other metal ions or under other buffer and *p*H conditions. The situation is further complicated by the acid-base properties of the metallochromic indicator itself. Obviously chelometric titrations with metallochromic indicators are actually much more involved than simple acid-base titrimetry. In the former as many as 13 competitive equilibria can be considered, whereas in most acid-base titrations only 3 or 4 competitive equilibria are usually involved. A full description of these competitive interactions has been given (*18*).

Many metallochromic indicators are blocked by traces of Cu, Co, Ni, and Fe, for example. This is often caused by their slow rate of reaction or by an unusually high stability of their complexes. Despite these limitations on the use of metallochromic indicators for end point detection, the experiments

described in this paper all utilize this means of end point detection because of its inherent simplicity and low cost and because of the fact that such blocking can often be avoided by appropriate conduct of the titration or addition of suitable marking agents. Some of the better titration conditions are summarized in Table 4.

Potentiometric Indication Using the Mercury Electrode

A mercury electrode may be employed for end point detection and also for the determination of metal-chelon stability constants (*16*, *17*, *19*, *21*, *25*, *27*, *35*). Because the end point can be detected in the titration of almost all metal ions and under a wide variety of solution conditions, this technique is not as restricted as metallochromic indicators.

A mercury electrode, in contact with a sample solution containing a metal ion, M^{+z}, and a small added quantity of mercury(II)-chelonate, HgY^{2-n}, exhibits a potential corresponding to the following half cell:

$$Hg|HgY^{2-n}, MY^{z-n}, M^{+z}$$

where Y^{-n} stands for the completely dissociated chelon. The following exchange equilibrium is established:

$$M^{+z} + HgY^{2-n} \rightleftarrows Hg^{+2} + MY^{z-n} \qquad (8)$$

The equilibrium constant expression for the exchange reaction can be solved for the concentration of $[Hg^{+2}]$ and its value substituted into the Nernst equation for the $Hg^{+2/0}$ couple. Hence the potential for this indicator electrode is (at 25°C)

$$E_{Hg} = E_{Hg}^{0} + 0.0296 \log \frac{[M^{+z}]\,[HgY^{2-n}]K_{MY}}{[MY^{z-n}]K_{HgY}} \qquad (9)$$

where K_{MY} and K_{HgY} correspond to the stability constants of the metal-chelon and Hg(II)-chelon complexes, respectively. The mercury electrode therefore responds directly to pM ($-\log$ [M]) in the vicinity of the end point where $[HgY^{2-n}]$ and $[MY^{2-n}]$ are constant and is applicable to the chelometric titration of metal ions in a manner analogous to the titration of acids with base using a glass electrode. Table 5 summarizes the different metal ions which may be successfully titrated with EDTA potentiometrically.

Most of the metal ions listed for the pH 4.0–5.5 group or the pH 8.0–10.0 group do not interfere with titrations performed at pH 2.0. Further, the alkaline earth ions do not interfere with titrations performed at pH 4.0–5.5. Thus, for example, a bismuth-cadmium-calcium mixture may be estimated by a stepwise procedure, the pH being increased after each end point. Halide, sulfide, cyanide, and similar substances that react with Hg_2^{+2} or Hg^{+2} interfere with the electrode response.

Other Techniques

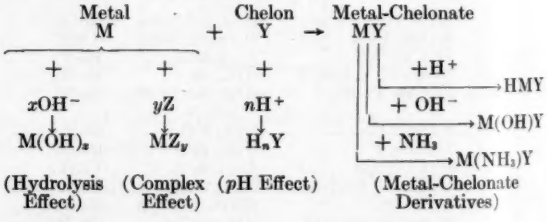
Photometric (*6*, *22*, *23*, *24*, *36*, *37*, *38*), conductometric (*7*), high frequency (*8*), thermometric (*10*), amperometric (*14*), and chronopotentiometric (*20*) methods have also been employed for detecting the end point of the chelometric titrations. Because of the rather limited applicability of these techniques (with the exception perhaps of the photometric one), no discussion of them is presented here.

Table 5. Potentiometric Titration Conditions of Metal Ions

pH	Buffer systems	Metals that may be titrated
1.5–2	HNO_3 or chloroacetic acid	*Direct titrations with EDTA solution:* Th^{+4}, Hg^{+2}, Bi^{+3} (avoid chloride contaminations)
4.0–5.5	Acetic acid or hexamethylenetetramine	*Direct titrations with standard EDTA sol.:* Sc^{+3}, Y^{+3}, La^{+3}, R.E.$^{+3}$, VO^{+2}, Mn^{+2}, Cu^{+2}, Zn^{+2}, Cd^{+2}, Hg^{+2}, Pb^{+2} *Back titration with Cu^{+2}, Zn^{+2}, Cd^{+2}, Hg^{+2}, or Pb^{+2} standard solutions:* Fe^{+3}, Ni^{+2}, Al^{+3}, Ga^{+3}, Zn^{+2}, Zr^{+4}, Hf^{+4}, Th^{+4}, Sc^{+3}, Y^{+3}, La^{+3}, R.E.$^{+3}$, VO^{+2}, Mn^{+2}, Cu^{+2}, Cd^{+2}, Hg^{+2}, Pb^{+2}, Bi^{+3}
8.0–10.0	Ammonia, triethanolamine, ethanolamine	*Direct titration with standard EDTA solution:* Mg^{+2}, Ca^{+2}, Sr^{+2}, Ba^{+2} (pH 10.0 and remove dissolved oxygen by bubbling nitrogen) Co^{+2}, Ni^{+2}, Cu^{+2}, Zn^{+2}, Cd^{+2}, In^{+3}, Pb^{+2} (for In^{+3} and Pb^{+2} add tartrate) *Back titration with Zn^{+2}, Ca^{+2}, Mg^{+2}, Cu^{+2}, or Cd^{+2} standard solution:* Sc^{+3}, Y^{+3}, La^{+3}, R.E.$^{+3}$, Hg^{+2}, Bi^{+3}, Co^{+2}, Ni^{+2}, Cu^{+2}, Zn^{+2}, In^{+3}, Pb^{+2}, Tl^{+3} (remove dissolved oxygen for larger break)

Pertinent Equilibria and Choice of Titration Conditions

For successful chelometric titrations, certain solution conditions—selection of pH, buffer type and concentration, and masking agents—must be established according to the metal ion titrated and the particular chelon used. The reaction of the metal ion with the chelon should, of course, be extensive for the metal ion being titrated and slight for the foreign metal ions. The various competitive equilibria are summarized (charges omitted):



pH Effect

Equation (10) shows that the hydrogen ion competes with the metal ion for the chelon. For this reason, metal ions which form weak chelonates can be titrated effectively only in alkaline solution. On the other hand, metal ions which form very stable chelonates can be titrated even in acid solution. The minimum pH at which metal ions of given stability can be titrated is calculated by taking into account the absolute stability constant of the chelonate and the acidity constants for the chelon. The acidity constants (that is, the stability constants of the proton complexes) can be defined as k_i and are given by the following expressions

$$\frac{[HY]}{[H^+]\,[Y]} = k_1, \quad \frac{[H_2Y]}{[H^+]\,[HY]} = k_2, \quad \frac{[H_3Y]}{[H^+]\,[H_2Y]} = k_3, \ldots \text{etc.} \qquad (11)$$

and the absolute stability constant by

$$\frac{[MY]}{[M]\,[Y]} = K \qquad (12)$$

Actually the unmetallized chelon exists in solution in several forms, the total stoichiometric concentration being designated as [Y]′

$$[Y]' = [Y] + [HY] + [H_2Y] + \ldots \qquad (13)$$

By substituting in the values for the acidity constants, expression (13) may be written

$$[Y]' = [Y]\,[H^+]k_1 + [Y]\,[H^+]^2k_1k_2 + \ldots + [Y]\,[H^+]^n\pi_nk_n \qquad (14)$$

The term [Y] can be factored from this expression

$$[Y]' = \alpha_Y[Y] \qquad (15)$$

where

$$\alpha_Y = 1 + [H^+]k_1 + [H^+]^2k_1k_2 + [H^+]^3k_1k_2k_3 + \ldots + [H^+]^n\Pi_nk_n \qquad (16)$$

The *p*H-dependent term α_Y, is extremely useful in all types of complex reactions facilitating the calculation of the concentration of the completely ionized chelon [Y] in terms of the stoichiometric concentration [Y]′. Because of the frequent need of its use, the numerical values as a function of *p*H are given in Figures 1 and 2 for the important chelons.

Substituting 15 into 12 yields

$$K = \frac{[MY]\,[\alpha_Y]}{[M]\,[Y]'} \qquad (17)$$

which can be rearranged to yield

$$\frac{K}{\alpha_Y} = K_{ett} = \frac{[MY]}{[M]\,[Y]'} \qquad (18)$$

where K_{ett}, the effective stability constant, denotes the *effective tendency for reaction between the metal and the chelon at any particular pH value.* The effective, apparent, or conditional stability constant necessary for a practical titration is given by

$$\log K_{ett} = T - \log C \qquad (19)$$

where C is the initial concentration of the metal ion to be titrated and T is an arbitrary "titration factor" whose numerical value depends on the mode of end point detection and the accuracy required. For end points obtained through extrapolation of straight lines (amperometric, conductometric, photometric) the value of T can be as low as 2 (2% reading error at half titration point). For potentiometric titration, the value of T should be above 4 and for color indicator titrations 5 or larger. With values of T greater than minimum, the end point sharpness improves markedly and the control of solution conditions will be less critical. From equations (18) and (19), the maximum value of log α, and thus the minimum *p*H, for a satisfactory titration can readily be calculated

$$\log \alpha = \log C + \log K - T \qquad (20)$$

Thus for the potentiometric ($T = 4$) titration of 0.01 M metal ions with EDTA, copper (log $K = 18.8$) requires a *p*H of 2.2 or higher, whereas calcium (log $K = 10.7$) requires 6 or higher. These calculations have neglected the "complex effect" and consequently the minimum *p*H values may be too low for many titration conditions.

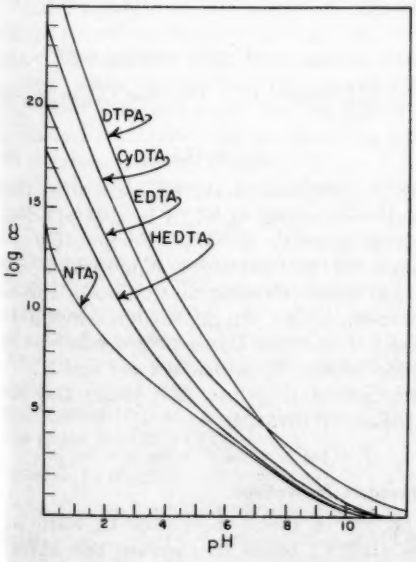


Figure 2. The dependence of log α on pH for NTA, HEDTA, EDTA, CyDTA, and DTPA.

Approximating Calculation of Log α

Because of the frequent use of log α values, a simple but approximate method for its calculation is useful. The answers agree very well with the values calculated by equation (16) except in regions where the *p*H is approximately equal to log k_i. Even there the answers are sufficiently accurate for most analytical purposes. The method is based on calculating α from the most predominant term of equation (16).

Consider the determination of log α at *p*H 5 for EDTA. The various species of EDTA which will be prevalent in solution at different *p*H's may be summarized

$$\underset{pH = 2}{H_4Y} \overset{\log k_4}{\rightleftarrows} \underset{2.76}{H_3Y} \overset{\log k_3}{\rightleftarrows} \underset{6.16}{H_2Y} \overset{\log k_2}{\rightleftarrows} \underset{10.26}{HY} \overset{\log k_1}{\rightleftarrows} Y \qquad (21)$$

Thus at *p*H 5 the prevalent form in solution is H_2Y and the value for log α is simply obtained by adding together the log k_i values greater than 5 (the *p*H) and subtracting the *p*H times the number of hydrogens of the prevalent form of the chelon. Thus log α (*p*H 5) = 10.26 + 6.16 − 2 × 5 = 6.42. At *p*H 7 the value for log α would be 10.26 − 1 × 7 = 3.26 and at *p*H 2.5, 10.26 + 6.16 + 2.76 − 3 × 2.5 = 11.68.

Hydrolysis Effect

The *p*H effect points to the use of alkaline conditions for most effective titrations. However, the hydrolysis of metal ions under such conditions lowers the effective stability constants as seen from equation (10) and counteracts the improvement gained by a decreased "*p*H effect." Because of the opposite influence of these effects, an optimum *p*H value exists for the titration of the given metal ion, the exact *p*H depending on the stability of the metal complex, the ease of hydrolysis

of the metal ion, and upon the pH-log α relationships of the chelon. The concentration of divalent metal ions (expressed as $p\mathrm{M}_h$) which decreases because of hydrolysis may be given most simply by:

$$p\mathrm{M}_h = pK_{sp} + 2p\mathrm{H} - 28 \qquad (22)$$

where K_{sp} is the solubility product constant of the metal hydroxide. The effective equilibrium constant for the reaction between metal hydroxide and the titrant then becomes

$$\log K_{eff} = \log K - \log \alpha - p\mathrm{M}_h \qquad (23)$$

For the chelon EDTA, the effective stability constant of mercury ($pK_{\mathrm{HgO}} = 25.5$), copper ($pK_{\mathrm{Cu(OH)_2}} = 18.8$), zinc ($pK_{\mathrm{Zn(OH)_2}} = 16.3$), and scandium ($pK_{\mathrm{Sc(OH)_3}} = 27$) were calculated as a function of pH and are illustrated in Figure 3.

The very striking result is that only a fraction of the absolute stability constant may actually be utilized for the reaction. The region between the dashed and the solid lines for each metal ion is lost because of the hydrolysis effect. The optimum pH value of the titration constant is obtained for mercury between the broad region from 3 to 5 but occurs at a rather well defined pH of 6 for copper and at 8 for zinc. At the optimum pH values the sequence of reaction between chelon and the metal ions is actually just the reverse predicted solely from the relative values of their absolute stability constants. Furthermore, the order of titration at pH 3 is just opposite to the order of titration at pH 10. Plots of this type for the various metals which complex with EDTA reveal that the largest effective stability constant obtainable is for scandium ($\log K_{eff} = 18.4$) although a number of other metal ions have a larger absolute stability constants than scandium ($\log K = 23.1$).

Knowledge of hydrolysis is now in a much more advanced stage than presented above, and detailed account can be taken of the various hydrolysis equilibria using relationships similar to those presented here.

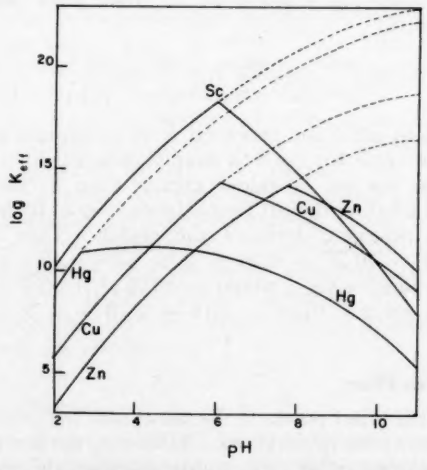


Figure 3. The effective stability constant for Sc^{+++}, Hg^{++}, Cu^{++}, and Zn^{++} as a function of pH.

Frequently titrations are performed in pH regions where the metal ion ordinarily precipitates as a hydrous oxide. Direct titrations of such metal precipitates is undesirable because titration reactions involving precipitates are extremely slow and, where metallochromic indicators are used, the end point is obscured by adsorption of the indicator on the precipitate. For these reasons precipitates are generally brought into solution prior to titration by the addition of a complexing agent. For the precipitate to dissolve, the complexing agent must increase the pM value slightly above the value, $p\mathrm{M}_h$, caused by hydrolysis. As a result, the log K_{eff} value will be diminished by its addition. In the ideal case the complexing action will be just sufficient to dissolve the precipitate and hence the log K_{eff} will be only slightly smaller than those plotted in Figure 3. Thus the conclusions drawn from Figure 3 are still valid.

The Complex Effect

The presence of complexing species other than chelon shifts the metal-chelonate equilibrium equation (10) to the left. Addition of a selective complexing agent often allows masking of certain metal ions, permitting titration of others. Furthermore, chelometric titrations are generally performed in the presence of buffer since the titration reaction ordinarily liberates hydrogen ions

$$\mathrm{M} + H_n\mathrm{Y} \rightarrow \mathrm{MY} + n\mathrm{H}^+ \qquad (24)$$

As most buffer chemicals are also complexing agents, they will decrease the value of log K_{eff}. For this reason, one must avoid the temptation of adding more buffer than is really necessary to maintain reasonably constant pH. The effect of buffer can be calculated from a knowledge of its stepwise formation constants, K_i, and from the acidity constant, k_i, of the buffer in a manner analogous to calculation of α for a chelon.

$$[\mathrm{M}]_t = [\mathrm{M}] + [\mathrm{MZ}] + [\mathrm{MZ_2}] + [\mathrm{MZ_3}] + \ldots \qquad (25)$$

$$= [\mathrm{M}] + [\mathrm{M}]\,[\mathrm{Z}]K_1 + [\mathrm{M}]\,[\mathrm{Z}]^2K_1K_2 + [\mathrm{M}]\,[\mathrm{Z}]^3K_1K_2K_3 + \ldots \qquad (26)$$

$$= [\mathrm{M}]\beta \qquad (27)$$

where $[\mathrm{M}]_t$ corresponds to the total stoichiometric concentration of unchelonated metal ion, and [Z] to the buffer,

$$\beta = 1 + [\mathrm{Z}]K_1 + [\mathrm{Z}]^2K_1K_2 + [\mathrm{Z}]^3K_1K_2K_3 + \ldots \qquad (28)$$

Thus β is [Z]-dependent. Because [Z] is pH-dependent,

$$[\mathrm{Z}]_t = [\mathrm{Z}]\alpha_{\mathrm{Z}} \qquad (29)$$

where $[\mathrm{Z}]_t$ is the stoichiometric concentration of unmetallized complexing agent, β is also pH-dependent for a given quantity of $[\mathrm{Z}]_t$. Fortunately a recent compilation of the pertinent equilibrium constants exists (*1*, *2*) hence allowing calculation of the complex effect in many cases. An approximate method to calculate log β is to follow the same procedure as for log α but employ $p\mathrm{Z}$ and $\mathrm{K_1}$ in place of pH and $\mathrm{k_1}$.

Substitution of (27) into (20) yields the condition for a satisfactory titration

$$T = \log K + \log C - \log \alpha - \log \beta \qquad (30)$$

Metal-Chelonate Derivatives

The ability of metal-chelonates to form acid derivatives (HMY) tends to increase the effectiveness of the titration of certain metal ions in acid solutions. This is especially prominent with DTPA, and this

chelon becomes actually superior to EDTA! For other chelons, the formation of the acid derivatives occurs only in pH regions where the metal chelonate is already too unstable for titration. Nevertheless, their effect often has to be taken into account since metal ions which form acid derivatives will tend to interfere more than one would expect solely from consideration of the log α-pH curve. Similarly the formation of $M(OH)_nY$ in alkaline solutions indicates enhanced metal-chelonate stability under such conditions. Some metal chelonates also form derivatives of the type MXY where X = NH_3, Cl^-, CNS^-, . . .

Factors Influencing Metal-Indicator Formation

Because most metallochromic indicators form 1:1 soluble chelates with metal ions, the algebra employed for calculating the various effects is identical in principle with that described for metal chelonates. Thus for MIn

$$\log K_{eff} = \log K_{MIn} - \log \alpha_{In} \qquad (31)$$

Similarly account must be taken of the occasional occurrence of complexes of the type MXIn. Thus the red Zn-Erio T complex reacts with ammonia to form purple Zn-NH_3-Erio T.

The colors of the various forms (e.g., H_2In^-, HIn^{-2}, In^{-3}) of the indicator are important. For Erio T, H_2In^- is pink, HIn^{-2} blue, and In^{-3} pink. Because most metal-Erio T complexes are pink, a significant color change (pink to blue) occurs only when the predominant form of unmetallized indicator is blue, HIn^{-2}. This places a further restriction on the pH conditions for titration.

Summary of Competitive Equilibria

pM-pH and pY-pH diagrams allow presentation in an easily digestible form of the competitive equilibria involved in chelometric titrations and are consequently quite useful in predicting suitable titration conditions. From such diagrams the sharpness of the end point can be quantitatively characterized and the effect of pH, buffer, type of chelon, and indicator can be readily seen (*18*). The pM-pH diagram is the ideal choice of presentation when comparing the applicability of various chelons and indicators for the titration of a single metal ion. If more than one metal ion is present, a pY-pH diagram is more suitable (*18*).

A typical pM-pH diagram is illustrated in Figure 4 and refers to the EDTA titration of 0.01 M calcium using Calcon. In this diagram, three main lines are drawn corresponding to various points in the titration (A at the beginning, B at 50% color change point, and C 100% beyond the end point). In pM-pH diagrams, the titration curve at constant pH proceeds from bottom to top in the sequence indicated and this accounts for the simplicity in its use. Line A, corresponding to the initial concentration, C, of the metal ion in the sample, is given by (32), (33), or (34):

$$pM_A = -\log C \text{ (no complex or hydrolysis effects)} \qquad (32)$$

$$= -\log C + \log \beta \text{ (complex effect)} \qquad (33)$$

$$= xp\mathrm{H} + pK_{sp} - x(14) \text{ (hydrolysis effect of } M(OH)_x) \qquad (34)$$

For 0.003 M calcium ion, equation (32) holds up to pH 12.5 and equation (34) is operative above this pH. As EDTA is added at pH 12.5, calcium is titrated and the pM value increases. Eventually line B, corresponding to the indicator color change, is crossed, thus marking the end point. The pM for this indicator line is given by equation (35) and is derived from equation (31) under the assumption that the indicator is present in equimolar concentrations in metallized and unmetallized forms,

$$[MIn] = [In]'$$
$$pM_B = \log K_{MIn} - \log \alpha_{In} \qquad (35)$$

Upon the addition of more EDTA, line C, corresponding to 100% excess of EDTA, is reached. The equation for this line is then given by substituting [CaY] = [Y]′ into equation (18)

$$pM_C = \log K_{MY} - \log \alpha_Y \qquad (36)$$

The right side of Figure 4 shows the resulting pM titration curve.

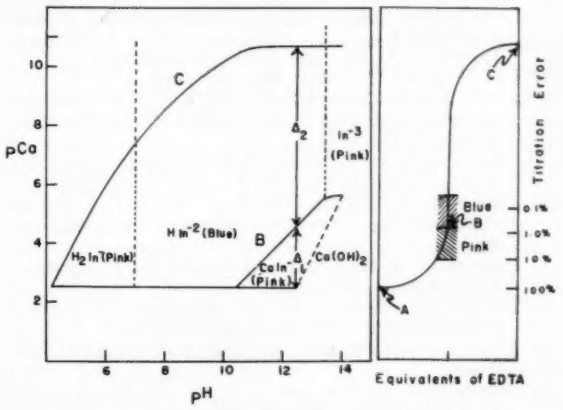


Figure 4. pM-pH diagram (left) and corresponding pM titration curve (right) for the titration of calcium with EDTA using Calcon as indicator.

The distances on the pM diagram denoted by Δ_1 and Δ_2 characterize quantitatively the sharpness of the end point. Values of Δ (in pM units) below 2 correspond to dragging end points. Values of Δ greater than 4 do not offer any significant improvement over the very sharp one obtained when Δ equals 4. Δ_1 designates the sharpness prior to the end point whereas Δ_2 corresponds to the sharpness beyond the end point. A symmetrical color change titration curve is obtained when $\Delta_1 = \Delta_2$ and the 50% color change point occurs at the equivalence point. The values of Δ_1 and Δ_2 vary significantly with pH and the vertical solid line at pH 12.5 indicates where Δ_1 and Δ_2 are both reasonably large. Under these conditions, the titration would be predicted as feasible and such is found in practice.

At pH 10.4 lines A and B intersect, or, more precisely, merge. The intersection point, as drawn in Figure 4, represents the pH at which the indicator complex is essentially completely dissociated by reaction with hydrogen ions, equation (10). Similarly, the intersection point of lines C and A indicates complete dissociation of the calcium-EDTA complex at pH 4.2 (disregarding the formation of hydrogen complexes).

At pH values, corresponding to the log k_i values of the indicator (vertical dashed lines), the unmetallized

indicator exists in two forms in equimolar concentration: at *p*H 13.5 as HIn^{-2} and In^{-3}, and at *p*H 7 as H_2In^- and HIn^{-2}. Thus if the *p*H of the sample solution containing indicator is varied, the following colors would be observed: *p*H up to 7, pink; *p*H 7 to 10.4, blue; above 10.4, pink. In contrast, for the over-titrated solutions the colors observed would be: *p*H up to 7, pink; *p*H 7 to 13.5, blue; *p*H above 13.5, pink. Hence the vertical solid line also indicates a *p*H where suitable color transition occurs at the end point. Further the equivalence point does not occur at 50% color change and the end point should therefore be taken when the last tinge of pink has just disappeared and the solution is pure blue.

A second paper will follow giving experiments suitable for laboratory classes and illustrating the methods of titration, ways of achieving selectivity, and precautions necessary for good results.

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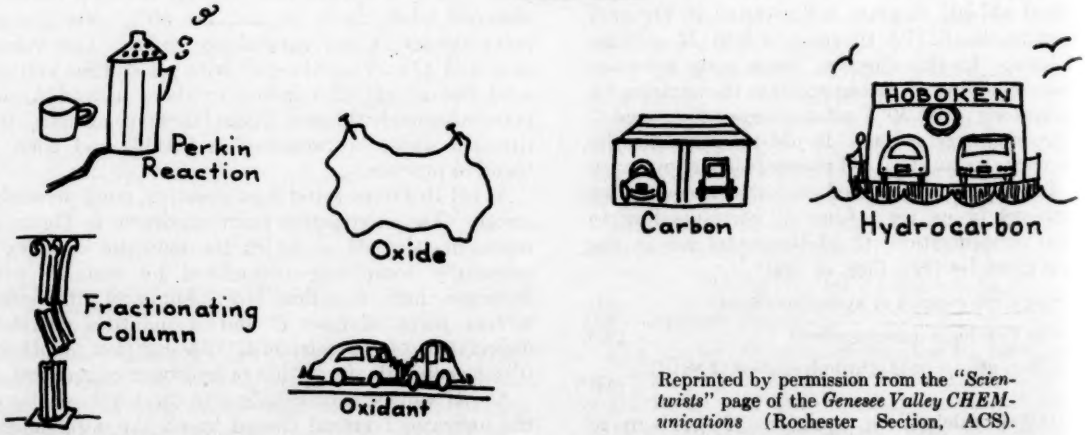
Chem Gems



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The Prehistory of the Periodic System of the Elements

If we wish to study the history of the periodic system, we must first investigate the state of the main scientific laws and theories in existence between 1780 and 1815 and the results of practical chemistry. The dating of the major laws of chemistry is common knowledge.[1]

1789 The law of the conservation of mass (Lavoisier) (*1*)
1807 The law of definite proportions (Proust) (*2*)
1808 The law of multiple proportions (Dalton) (*3*)
1808 The law of Gay-Lussac concerning the relations of the volumes of the gases in a chemical reaction (*4*)
1811 The hypothesis of Avogadro (*5*)

The theory in which Richter (*6*) recorded his principles of stoichiometry dates from 1792–93, and Dalton's (*3*) atomic theory was set up in 1803 and published in 1808. Supplementing these is the molecular theory of Avogadro, set up in 1811. Dalton's (*7*) first table of atomic weights[2] was published in 1805 (set up in 1803).

This first table contained only 6 elements, but as early as 1808 Dalton published a table with 20 elements, which was supplemented in 1810. Atomic weight tables were published, too, by three other scientists: Thomson (*8*) in 1810, Wollaston (*9*) in 1814 and Berzelius (*10*) in 1815, of which Berzelius' table contained the greatest number of elements, viz., 43 out of 48 isolated elements.

With this background in mind one might suppose that in the second decade of the 19th century the time had come to set up at least a large number of groups of elements with analogous properties, the atomic weights of which form arithmetical progressions. Döbereiner was the only one, however, to set up—in 1817 and 1829—his triads, groups of three elements whose properties are analogous and whose central element's atomic weight is the mean of the atomic weights of the other two. Scientists struggled with the problem of the analogies between elements as regards their atomic weights until the middle of the 19th century. Today it is hardly possible to imagine that half a century was to pass before the theories and the results of modern chemistry could be used as the basis for the system of the elements. Perhaps this long delay may be ascribed to the number of elements undiscovered at that time. In 1817 only 50 elements were known, but if we bear in mind that most of the rare earth metals—which could not then be ranged correctly in a simple system—were among those elements still undiscovered, we may safely assume that this number was quite sufficient to carry through a classification for their properties had they been adequately examined.

The cause of the difficulties seems to have been the lack of a definite conception of atomic weight, which is very closely connected with the definitions of molecules and atoms. The prevailing confusion about atomic weight and of what is now called "valency," caused by underestimation and misinterpretation of the laws and theories mentioned above, made it impossible to arrive at an unequivocal list of atomic weights, notwithstanding the fact that able scientists like Berzelius, Thomson, and Wollaston had determined atomic or equivalent weights. For example, Dalton did not accept Gay-Lussac's law on experimental grounds[3] and since it was not in accordance with his diffusion theory.[4] Neither did he know Avogadro's explanation of this law, whereas Avogadro's hypothesis in particular could have corroborated Dalton's theories. The latter, however, was a follower of Newton's in the belief that similar particles repel each other, while Avogadro assumed the existence of diatomic molecules of various gases.

What attitude was adopted by the great Berzelius in this matter? Could he not have provided a solution as a result of his many and accurate quantitative experiments? From his first publications (*11*) in 1810 and 1812 it appears that he did realize the importance of Richter's equivalent weight rules, Dalton's law of multiple proportions, and Proust's law.[5] In these years, however, he had not yet formed a theory concerning the composition of compounds although he had analyzed a great many of these. Dalton's atomic theory obviously did not mean much to him either. And though Berzelius, himself a true-born experimenter, immediately shared Gay-Lussac's conclusion as to the proportion of volumes of the gases that participate in a reaction, he did not, like Avogadro,[6] assume diatomic molecules. Therefore, it is easy to understand how in 1827 Berzelius had to enter into a scientific duel with

Presented before the Section for the History of Chemistry of the Royal Dutch Chemical Society on October 1, 1956. Published by kind permission of the Society.

[1] The dates indicate the year of publication, not that of formulation. By adopting this method we mean to indicate that through these publications the above-mentioned laws and theories were accessible to all.

[2] We have employed the term "atomic weight" even though the scientist in question used a different term, i.e., equivalent weight.

[3] Conversely Gay-Lussac does not use Dalton's atomic theory in 1808.

[4] According to some scientists—Dalton and Berzelius (up to 1819)—it follows from Gay-Lussac's law that, e.g., nitrogen and oxygen atoms are of the same size, which should make a reaction between these two elements impossible in Dalton's diffusion theory.

[5] This law met with severe opposition from Berthollet.

Dumas and to take up a fight with Gmelin and Gerhardt. Gmelin was an advocate of Avogadro's theory which he used to determine the value of atomic weights from the value of vapor densities[7] while Gerhardt consequently applied the hypothesis to organic gases, a method expanded by Laurent.

Before going on we would do well to consider the theories of some scientists on the absolute values of atomic weights and molecular structure. Dalton's ideas about the combination between atoms were quite straightforward; most compounds of two elements are of the AB type, while if there are more combinations of two elements, these have the formula AB_2, A_2B, etc. Thus he gave to water the formula HO ("translated" into the customary formula), hence the value 5.5 for the atomic weight of oxygen. Besides being a deviation in principle, this value points to an inaccurate experiment. Other values, too, published in 1805 in his table of atomic weights called "The relative weight of the ultimate particles of gaseous and other bodies," deviate considerably from the present values: C = 4.3, N = 4.2, P = 7.2, and S = 14.4. In 1808 quoted values for these atoms are a good deal more accurate (*3*); and the atomic weights table contains additional elements, too, with a total of 20.

In Berzelius' (*10*) table of atomic weights, published in 1815, many metals are given a value of twice the present one. Berzelius assumed that one atom of metal could combine with 1, 2, 3, 4, etc., atoms of oxygen. As a result the formulas of the iron oxides became FeO, FeO_2, FeO_3, FeO_4, etc. As he found that the quantities of oxygen which had combined with an equal mass of iron were in the ratio 2:3:4, Berzelius believed that the oxide FeO still had to be discovered. According to this reasoning, the atomic weight of iron would be 112. As early as 1818 he realized his errors and believed that instead of the above-mentioned series of iron oxides there are three known oxides with formulas FeO, Fe_2O_3, FeO_2. This view was consistent with 56 for the atomic weight of iron. In his list of atomic weights of 1826 we find the correct value for the atomic weight of most elements. Berzelius made use of the theory of isomorphism and of the law of Dulong and Petit (1819, 1820) for the determination of the atomic weights. Only the atomic weights of the alkali metals and other univalent elements (e.g., silver which in 1815 had had an atomic weight which was four times too high) now still had a value which was twice too high. Berzelius gave the oxides of these metals the formula MO. A year later, in 1827, Gmelin (*13*) published the correct values for the atomic weights of the univalent metals. He, too, accepted the formula MO for all lowest metal oxides, including water, which he gave the formula HO. We can now understand how he obtained an atomic weight 8 for oxygen. With this value as standard the divalent, trivalent, and quadrivalent metals were listed as having an atomic weight which was two, three, or four times too small (thus: Mg = 12, Al = 9, Zr = 22.4).

In 1828 Dumas (*14*) set up a somewhat more comprehensive list than that of Berzelius. He, too, chose O = 16 so that the univalent metals were given atomic weights twice too high.

Although he constantly talked about the term atomic weight, Berzelius also used equivalent weight. This, together with the various versions of the formulas advocated by different scientists, brought the confusion to its climax.

In the thirties Gay-Lussac, Liebig, and Gmelin reach back to the equivalent weights of Wollaston. These actually were supported by Faraday's research of 1834 on the subject of electrolysis. Also on account of the still vague understanding of valency, it was not until the forties, when the opposition to Berzelius had culminated, that a further step was taken in the direction of solving the atomic weight problem. In 1843 Gerhardt (*15*) altered some of Berzelius' atomic weights in such a way that the atomic weights of the alkaline metals became correct, but those of the divalent metals were halved. His starting point was the formula M_2O for the lowest metal oxides.

We must now consider the atomic weights which some precursors of the periodic system had at their disposal. Döbereiner (*16*) could have used Berzelius' atomic weights or those of Wollaston, Dalton, or Thomson for his discoveries of the year 1817. It appears that he used approximately Wollaston's values for the equivalent weights of calcium, strontium, and barium. For his first triad he used the oxides of these elements. When we compare this triad (after recalculation of the atomic weights on the basis O = 16 instead of Wollaston's O = 7.6) with those which we can now set up, we very clearly see the close agreement:

$$\text{Döbereiner's triad: } SrO = \frac{CaO + BaO}{2} = 107 = \frac{59 + 155}{2}$$

$$\text{Present-day triad: } = 105 = \frac{56 + 153}{2}$$

In 1829 Döbereiner (*17*) made use of the atomic weights which Berzelius determined in 1826 and was able to add a few more triads. One of these was:

$$Br = \frac{Cl + I}{2} = \frac{35.470 + 126.470}{2} = 80.470$$

(This last value is a printer's error and should be 80.970.) The atomic weight which Berzelius calculated for bromine was 78.383.

Even after this publication the scientific world continued to shun this particular subject. Döbereiner was truly far ahead of his time. Only Gmelin (*13*) set up some relationships in 1827. In 1843 he appeared to be acquainted with Döbereiner's work (*18*). In that same year Gmelin himself added a further number of relationships.

In 1850, 33 years after the first publication by Döbereiner—who died in 1849—Pettenkofer (*19*) once again touched on this subject. By that time the situation with regard to the atomic weights had changed. Most of the atomic weights which Berzelius then used corresponded to the present ones. Pettenkofer, when setting up his relationships, made good use of Berzelius' table of atomic weights (*20*) of 1845.

Between 1850 and 1860 many relationships between the atomic weights of analogous elements were set up. Dumas (*21–26*), Gladstone (*27*), Cooke (*28, 29*), Lenssen (*30, 31*), Odling (*32*), and Mercer (*33*) prepared

[6] Up till 1825 not a single scientist paid any attention to Avogadro's work. Berzelius did not enter into his theories until 1833. His name was not even mentioned in Kopp's (*12*) "Geschichte der Chemie," published in 1844.

[7] When it appeared, however, that vapor densities determined at high temperatures did not agree with the theory of Avogadro, he abandoned this theory.

the way which the discoverers of the periodic system were to follow some years later. It was chiefly Odling (*32*) and Dumas (*26*) who in 1857 and 1858 saw some connection between what had formerly been considered independent groups of analogous elements. The first person to set up the periodic system was the French mineralogist Béguyer de Chancourtois (*34*). He called it the "vis tellurique." He was aware of Dumas' research but would not have been able to set up an elaborate system in 1862 if Cannizarro had not held his illuminating discourse at the Congress of Karlshuhe in 1860 on the determinations of the atomic weights and of the formulas of the compounds, using Avogadro's hypothesis as basis.

The year 1860 must be considered as one of the highlights in the history of theoretical chemistry. The way had been prepared. The first who had set foot on it, de Chancourtois, (*34, 35, 36*) was immediately followed by Newlands (*37–45*), Odling (*46, 47*), Hinrichs (*48*), Meyer (*49, 50, 51*), and Mendeleev (*52–56*). Each had an important share in establishing the periodic system of the elements. We believe that we must consider these six scientists to be independent discoverers.

Yet de Chancourtois was not the first scientist to see a great connection. Strecker (*57*) in 1859 had already remarked,

> Es ist wohl kaum anzunehmen, dasz alle hervorgehebenen Beziehungen zwischen den Atomgewichten (oder Aequivalenten) in chemischen Verhältnissen einander ähnlichen Elemente blosz zufällig sind. Die Auffindung der in diesen Zahlen durchblickenden gesetzlichen Beziehungen mussen wir jedoch die Zukunft überlassen.[8]

In 1852 Faraday (*58*) was already enthusiastic about Dumas' work. Faraday comes to the conclusion that it might very well be possible that a new law concerning the elements might prove to exist, for "... when we come to examine the combining powers of the three, as indicated by their respective equivalents or atomic weights, the same mutual relation will be rendered evident. This circumstance has been made the basis of some beautiful speculations by Mr. M. Dumas—speculations which have scarcely yet assumed the consistence of a theory, and which are only at the present time to be ranged amongst the poetic illuminations of the mental horizon, which possibly may be the harbinger of a new law."

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[8] It can hardly be assumed that all the previously mentioned relationships between the atomic weights of similar elements in compounds are merely accidental. But the discovery of the laws emerging from these numbers we will have to leave to the future.

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Humphry Davy and the Elementary Nature of Chlorine

It is commonly stated in beginning chemistry texts and in histories of chemistry that Humphry Davy was the first to demonstrate the elementary nature of chlorine. However, these sources usually omit two points essential to the understanding of the historical significance of Davy's work: first, the conceptual and experimental difficulties associated at that time with the establishment of any substance as an element, and second, the effect that the establishment of chlorine as an element had on contemporary chemical theory. It is the purpose of this paper to give a brief account of this episode in the development of chemical thought, not only because it is inadequately treated in most available sources, but also because it illustrates certain philosophical problems of scientific knowledge which are still present in modern science.

The significance of Davy's work on chlorine lies in its relationship to certain views established by Lavoisier and his contemporaries of the late eighteenth century chemical revolution. By the first decade of the nineteenth century, it was becoming increasingly clear that Lavoisier's most significant contribution was his insistence on the quantitative measurements which would allow the development of chemistry into a more nearly exact science. Though Lavoisier can hardly be credited with originating quantitative procedure, his successful use of it in clarifying the nature of combustion accelerated its general acceptance as standard chemical practice. The particular aspect of this quantitative experimental view which interests us here is his definition of the chemical element. To Lavoisier a chemical element was any substance which had not yet been decomposed. The application of such a definition must involve quantitative analytical procedures circumscribed by the law of conservation of mass. But even so, the results were tentative and Lavoisier's list of elements was subject to deletions and additions. If a substance failed to yield to determined efforts to decompose it, it was assigned to the list of elements. But no amount of experimental evidence could prove that a substance could not be decomposed. The conditional nature of the list of elements was widely recognized and felt to be philosophically unsatisfying. As a result, chemists of the early nineteenth century frequently raised the question as to the elementary nature, not only of new substances, but of some of the old ones as well. Charcoal, sulfur, and phosphorus among the old, and palladium, sodium, and potassium among the new, were some of the elements which were seriously considered by respectable chemists to be compound.

In addition to the general philosophical difficulties associated with all the elements at this time, the study of chlorine involved two others which stem from the central role given to oxygen in the generally accepted chemical theory. Beginning with the writings of Lavoisier, oxygen was considered the sole agent responsible for the support of combustion, and it was regarded as the essential ingredient of all acids, as the etymology of its name implies. Other substances which supported combustion were believed to do so because they contained oxygen, for example, nitrous oxide and oxy-muriatic acid gas (chlorine).

Acids were formed by the combustion of certain acidifiable substances, the most important of which were sulfur and phosphorus. Though acids were commonly used in water solution, the water was considered only as a solvent and no significant interaction between the acid (anhydride) and the water was postulated. Acids, either dry or in solution, reacted with bases or earths (metallic oxides) to form salts. According to this scheme, all salts contained oxygen and such a thing as a binary salt was inconceivable.

Consistent with the oxygen theory, muriatic acid (HCl) was thought to be composed of oxygen and some as yet unisolated substance, presumably an element. Muratic acid reacted with pyrolusite (MnO_2), which was known to contain an excess of oxygen, to produce a gas thought to be a combination of the excess oxygen with the muriatic acid. This gas was accordingly known as oxy-muriatic acid gas (chlorine). Since this gas presumably contained more oxygen than muriatic acid, it should have possessed stronger acid properties than muriatic acid, but the facts were just the reverse. Because of this inconsistency between fact and theory, Lavoisier expressed some uncertainty about the composition of oxy-muriatic acid gas. None the less, the composition implied by the name remained the generally accepted one up to 1810.

Prior to Davy's work on chlorine in 1810, a number of workers had attempted to confirm the presence of oxygen in muriatic acid and in oxy-muriatic acid gas, but without success. The failure of these two substances to conform to the oxygen theory of acids constituted the most obvious weak point of that theory, but it was also widely known that hydrogen cyanide and hydrogen sulfide were weakly acidic though containing no oxygen. Though generally accepted, the oxygen theory of acids was very much an open question in the early nineteenth century.

Davy reasoned that if oxy-muratic acid gas (chlorine) were composed of muriatic acid and oxygen, charcoal would be expected to react with the oxygen and liberate the muriatic acid. Previous attempts by a number of workers to carry out this reaction had given ambiguous results, largely because of the organic

materials normally present in the charcoal. Davy heated his charcoal electrically to drive off all volatile matter and then exposed it at a white heat to a stream of dry oxy-muriatic acid gas, but there was no reaction. This result led him "to doubt of the existence of oxygen in that substance, which had been supposed to contain it above all others in a loose and active state."

Davy then carried out several additional experiments, each designed to separate the oxygen from the muriatic acid. When phosphorus was treated with chlorine, he obtained a mixture of phosphorus and phosphoric chlorides. Separating the phosphoric chloride, which according to the current interpretation was a compound of phosphoric acid (P_2O_5) and muriatic acid, he added to it some dry ammonia gas. It was intended for the ammonia to react with the muriatic acid to form ammonium muriate (NH_4Cl) and leave the phosphoric acid, but the resulting product possessed none of the properties of either expected compound. The product was a solid white mass, unaffected by any reagent except hot concentrated alkali. (This was probably a phospham polymer, and Davy was much puzzled that its properties were so different from those of its ingredients.)

Metallic potassium plus oxy-muriatic acid gas gave a dry salt (KCl). When the gas was added to potassium oxide, the same salt was produced and oxygen liberated. Though he admitted that the oxygen might have come from the oxy-muriatic acid gas, he pointed out that "It is contrary to sound logic to say, that this exact quantity of oxygen is given off from a body not known to be compound, when we are certain of its existence in another."

In another experiment, he was unable to detect any change in dry oxy-muriatic acid gas after passing electric sparks through it with "strong explosions" for several hours.

As a result of these and many other unsuccessful attempts to detect oxygen in oxy-muriatic acid gas, Davy pointed out that the behavior of this substance could more easily be explained by assuming it to be one of the elements, or as Davy cautiously called them, the undecompounded bodies. He also suggested that the name be changed to chlorine, basing the choice only on the color of the gas. "Should it hereafter be discovered to be compound, and even to contain oxygen, this name can imply no error, and cannot necessarily require change."

Davy's subsequent discovery of chlorine dioxide provided further evidence which was most easily explained by assuming chlorine to be an element. "If the power of bodies to burn in oxy-muriatic gas depended upon the presence of oxygen, they all ought to burn with much more energy in the new compound. . . ." This was not the case.

His final argument was based on the fact that phosphorus oxymuriate (PCl_5) reacted with oxygen at a red heat to form phosphoric oxide and liberated the oxy-muriatic acid gas. "Now if oxygen existed in the oxy-muriate of phosphorus, there is no reason why this change should take place. On the idea of oxy-muriatic gas being undecompounded, it is easily explained. Oxygen is known to have a stronger attraction for phosphorus than oxy-muriatic gas has, and consequently ought to expel it from this combination."

In retrospect, it seems just to give Davy credit for establishing the elementary nature of chlorine, but Davy himself made no such claim. By his own standards, he had shown only that he had been unable to detect any oxygen in the substance, and that its properties were most easily explained by assuming it to be simple. "As yet we have no right to say that it has been decompounded; and as its tendency of combination is with pure inflammable matters, it may possibly belong to the same class of bodies as oxygen." Davy here for the first time suggests that oxygen is not unique in its chemical properties, as had been assumed, and that chlorine is to be regarded as an analogous substance. He marshalled considerable evidence to support this idea. The fact that chlorine supported combustion Davy explained by giving a broader definition to that process, stating that the emission of heat and light, so characteristic of combustion is ". . . merely the result of the intense agency of combination," and is not confined to those reactions involving oxygen. He cited as examples the combinations of sulfur with certain metals which also produce heat and light, and ". . . such an effect might be expected in an operation so rapid, as that of oxy-muriatic acid upon metals and inflammable bodies."

In regard to the fact that chlorine, though containing no oxygen, formed a strong acid when combined with hydrogen, Davy said, "May it not in fact be a peculiar acidifying and dissolving principle, forming compounds with combustible bodies, analogous to acids containing oxygen. . . On this idea muriatic acid may be considered as having hydrogen for its basis and oxy-muriatic acid for its acidifying principle."

The unique role of oxygen in chemical theory had forced chemists to consider chlorine as containing oxygen. That is, chlorine was similar to oxygen because it contained oxygen. The experimental evidence provided by Davy made this view difficult to accept and in its place he suggested that chlorine should take its place beside oxygen as another substance capable of supporting combustion and of forming acids. Davy also emphasized that chlorine like oxygen was strongly electronegative and that these two elements were the only ones known to be liberated by electrolysis at the positive wire. If all this destroyed the oxygen theory, so much the worse for the theory, for to Davy, chemistry was too young a science to be developing theories which at this stage could only lead to rigidly established error.

Other chemists, however, were justifiably reluctant to give up the advantages of a unifying theory on the strength of the negative evidence derived from only one substance. They could and did reserve judgment about chlorine while still using the oxygen theory in many of its other applications.

The discovery of iodine in 1811 and the subsequent investigation of its properties (by Gay-Lussac and by Davy) provided another substance so similar to chlorine that it had to be classed with chlorine and oxygen among the electronegative elements. Davy's investigation of the compounds of fluorine furnished still another member of this class. As a result of this accumulation of evidence, Davy's views ultimately prevailed.

Davy succeeded not only in establishing chlorine as an element, but also in undermining Lavoisier's oxygen

theory. In recognizing the inconsistency between the properties of chlorine and the requirements of that theory, he characteristically attacked the problem by gathering new experimental evidence and further demonstrated the inadequacies of the theory. Though Davy's satisfaction lay in this re-affirmation of the primacy of experiment over speculation, he had also cleared the ground for the later development of the hydrogen theory of acids and still broader concepts of oxidation.

Bibliographic Note

Those who wish to read the original literature on which this paper was based will find most of the pertinent material collected in two Alembic Club Reprints (University of Chicago Press, 1906). Reprint Number 13, "The Early History of Chlorine," contains translations of selected passages from the writings of C. W. Scheele, who in 1774 first prepared chlorine and described it as dephlogisticated marine acid air; and from C. L. Berthollet (1785) and L. B. Guyton de Morveau (1787), who developed the concept that the new gas was oxy-muriatic acid gas, in conformity with Lavoisier's oxygen theory of acids. The final section in this Reprint is from later (1809) writings of J. L. Gay-Lussac and L. J. Thenard who, after extensive investigation of the properties of chlorine just prior to Davy's work, decided that the interpretation of Berthollet was still to be preferred. They did mention, however, that the gas might be considered an element, though they rejected this interpretation for themselves.

Reprint Number 9, "The Elementary Nature of Chlorine," contains nearly all of Davy's writings which deal with chlorine, including some which antedate his conviction that chlorine contained no oxygen. Thus the transformation of Davy's views regarding the nature of chlorine, and the experimental evidence supporting it, are clearly and chronologically given in his own words.

The conscientious reader of these Reprints will find his time rewarded by an increased respect for those chemists who faced the tremendous difficulties of a time when the list of elements had no independent confirmation from the periodic table or from Moseley's law, and when the systematic use of quantitatively based arguments had hardly been established.

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Laboratory Synthesis of a Heterocyclic Compound

The preparation and reactions of heterocycles are often neglected in the course in general organic chemistry. That time is a major factor in determining which topics to omit can hardly be debated. However, lack of time in the laboratory need not be a reason for missing the opportunity to give students practical experience with heterocyclic compounds if rather short experiments can be developed. Short preparations of ϵ-caprolactam, fluorescein, and cyclic anhydrides and imides may be found in a number of laboratory manuals; excellent longer experiments are included in the laboratory manuals of Mackenzie[1] and of Robertson.[2] Mackenzie's preparation of phenylmethylpyrazolone requires two and one-half hours and his preparation of hydantoin requires two hours. Robertson's preparation of quinoline requires eight to ten hours.

We have developed a test tube experiment which requires approximately 45 minutes and which, moreover, serves to introduce to the student a valuable research technique. Because of the proximity of the melting points of *o*-nitroaniline and of benzofurazan oxide, the fact that both contain the same elements, and their similarity in color (orange and yellow), it becomes feasible to demonstrate non-identity of starting material and product by mixture melting point. The procedure for the preparation of benzofurazan oxide is an adaptation of the method of Mallory.[3]

Preparation of Benzofurazan Oxide. Add to 15 ml of a 10% solution of potassium hydroxide in ethanol contained in a 25- × 200-mm test tube one gram of *o*-nitroaniline. Warm the mixture slightly until the solid dissolves. While swirling the solution, add dropwise a commercial bleaching solution until precipitation is complete (about 30 ml of "Chlorox" is necessary, for example). Remove the yellow crystals by suction filtration. Dissolve the moist solid in 10 ml of ethanol, filter if the solution is turbid, and add 20 ml of water dropwise. Remove the solid by filtration and allow the crystals to dry overnight. Determine the melting point and percentage yield of the product. Observe the melting behavior of a mixture of the product and *o*-nitroaniline.

In a typical performance the melting point of the *o*-nitroaniline (Eastman product, not recrystallized) was 69.5–71°, the melting point of the benzofurazan oxide was 66–67.50°, the melting range of a 50:50 (weight) mixture of the starting material and product was 38–61°, and the yield was 40%. More of the product could be recovered from the ethanol-water filtrate, if desired.

[1] MACKENZIE, C. A., "Experimental Organic Chemistry," Prentice-Hall, Inc., Edgewood Cliffs, N. J., 1955, pp. 175, 176.

[2] ROBERTSON, G. R., "Laboratory Practice of Organic Chemistry," 3rd ed., The Macmillan Co., New York, 1954, p. 330.

[3] MALLORY, F. B., in "Organic Syntheses," Vol. 37, edited by J. CASON, John Wiley & Sons, Inc., New York, 1957, p. 1.

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A. W. Williamson and the Impersonal Passive

Students, authors, and editors at times find considerable difficulty in eliminating the personal pronoun "I" or "me" from scientific writing. It is, therefore, of some interest to find a paper by an eminent scientist published both in the impersonal and the personal form. Alexander Williamson's classic paper on the preparation and constitution of ethers was first delivered as a lecture before the British Association and was published in the *Philosophical Magazine* and *Journal of Science*.[1] In this paper personal pronouns abound.

In the early years of the Chemical Society of London, that organization published the *Quarterly Journal of the Chemical Society*. The Council of the Society had instructed the editors to reprint important articles from other less accessible journals. Williamson's paper[2] appeared there under the original title, "Theory of Etherification," and with only a footnote stating that the article had been "reprinted" from the above-mentioned source. In fact the articles are identical except for the conversion of the personal to the impersonal form and minor changes in the spelling, such as in the names of alkyl radicals—e.g., from methyle to methyl (still pronounced *meethile* in England).

The change was *not* due to editorial policy, for other articles in the same and succeeding journals still used the personal pronouns. There did seem, however, to be a trend toward the impersonal during those years, evidenced, for instance, by the use of "the authors" for the much simpler "we."

One may speculate as to the reasons for the change to the impersonal which has now become almost universal in technical journals. It is clearly not due to a great increase in humility, for the scientists of the Renaissance were most conscious of merely discovering God's handiwork, yet were prolific in their use of personal pronouns. Probably the change is due to the fact that in the early period of modern science the novel element was not so much the discovery of new facts, as the new way of looking at natural phenomena. Galileo's "Dialogues" present very few new experimental facts. The major purpose of the dialogues is to contrast the Aristotelian viewpoint of Simplicius, concerned with final causes, with Galileo's own view. This new viewpoint, seeking for causal relations among natural phenomena rather than ultimate reasons, the "how" rather than the "why," has now become universal in science. The mode of thought of the investigator is, therefore, no longer of interest in a journal devoted to the reporting of scientific results.

It is ironic that this transformation in the form of scientific writing should become established in a century that is intensely interested in the psychology of discovery and invention. Scientific journals give practically no clue in our time as to the paths by which discoveries are made or new ideas emerge. To discover these paths we must usually wait till a scientist receives the Nobel prize or other honor and is willing, from his new eminence, to reveal a little of the unpublished history of his scientific contributions.

Some parallel passages of the two Williamson papers are set out below:

[1] WILLIAMSON, A. W., *Phil. Mag.*, [3], **37**, 350–6 (1850); reprinted in WILLIAMSON, A. W., "Papers on Etherification," Alembic Club Reprint No. 16, E. and S. Livingstone Ltd., Edinburgh, 1949, pp. 7–17.

[2] WILLIAMSON, A. W., *Quart. J. Chem. Soc.*, **4**, 106–12 (1852).

Philosophical Magazine (1850)

My object in commencing the experiments was to obtain new alcohols . . . .

I commenced with common alcohol . . . .

. . . but, to my astonishment . . . .

Now this result at once struck me as being inconsistent with the higher formula of alcohol, for if that body contained twice as many atoms of oxygen as are in aether, I ought clearly to have obtained a product containing twice as much oxygen as aether does.

. . . but I have not felt myself justified in doing so . . . .

. . . I devised a further and more tangible method of arriving at a conclusion.

My task is now to explain . . . .

I must beg leave to disclaim for it the title of innovation; for my conclusion . . . .

I believe this explanation coincides . . . .

. . . my explanation . . . .

In using the atomic theory, chemists have added to it of late years an unsafe, and, as I think, an unwarrantable hypothesis, namely that the atoms are in a state of rest. Now this hypothesis I discard, and reason upon the broader basis of atomic motion.

Quarterly Journal (1852)

The following experiments were made with the view of obtaining new alcohols. . . .

The process was first tried with common alcohol. . . .

. . . but, contrary to expectation. . . .

Now this result at once appeared to be inconsistent with the higher formula of alcohol; for if that body contained twice as many atoms of oxygen as are in ether, the product ought clearly to have contained twice as much oxygen as ether does.

. . . but the author has not felt himself justified in doing so. . . .

. . . a further and more tangible method of arriving at a conclusion was devised.

The next thing to be done is to explain. . . .

. . . the author begs leave to disclaim for it the title of innovation; for the conclusions here deduced. . . .

This explanation coincides. . . .

The preceding explanation. . . .

In using the atomic theory, chemists have added to it of late years an unsafe and perhaps unwarranted hypothesis, namely, that the atoms are in a state of rest. This hypothesis the author of the present paper discards and reasons upon the broader basis of atomic motion.

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The Iodoform Reaction

Qualitative organic analysis offers unique opportunities for the teacher to challenge students to develop a facility at interpretation. The iodoform reaction offers an excellent example for the type of analytical thinking which should be required. This paper presents a review of the theoretical basis of the reaction and a consideration of the factors which influence its utility in an analysis procedure. Some suggestions on means of obtaining improved specificity are included.

Mechanism of the Reaction

The formation of iodoform from ketones is believed to occur through the following steps (*1*, *2*):

$$RCOCH_3 + OH^- \rightarrow RCOCH_2^- + H_2O \quad \text{(slow, rate determining)} \qquad (1)$$

$$RCOCH_2^- + HIO \rightarrow RCOCH_2I + OH^- \quad \text{(fast)} \qquad (2)$$

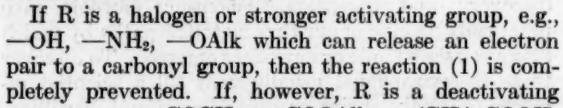
The electron withdrawing inductive effect of the iodine in $RCOCH_2I$ facilitates rapid successive iodination to $RCOCI_3$ which is then rapidly hydrolyzed,

$$RCOCI_3 + OH^- \rightarrow RCOO^- + CHI_3 \qquad (3)$$

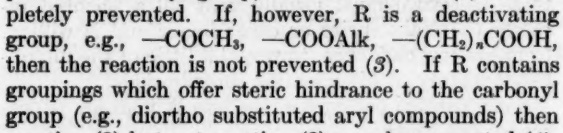
In the formation of iodoform from alcohols of the formula $CH_3CH(OH)R$ there is an additional step, the initial oxidation to CH_3COR.

If R is a halogen or stronger activating group, e.g., —OH, —NH_2, —OAlk which can release an electron pair to a carbonyl group, then the reaction (1) is completely prevented. If, however, R is a deactivating group, e.g., —$COCH_3$, —COOAlk, —$(CH_2)_nCOOH$, then the reaction is not prevented (*3*). If R contains groupings which offer steric hindrance to the carbonyl group (e.g., diortho substituted aryl compounds) then reaction (3) but not reaction (2) may be prevented (*4*). When R is the aryl group, activating groups in the nucleus will favor preferential iodination over the immediate formation of iodoform. The deactivating effect of the iodine atoms so substituted tends to balance the effect of the activating groups leading to little alteration of the rates of steps (1) and (3). Deactivating groups in the nucleus however facilitate these two steps directly.

It is possible to observe experimentally an inversion of the order in the rates of steps (1) and (2). If we consider the iodoform reaction with *m*-nitro acetophenone we have



(4)



(5)

Step (4) will be more rapid than with acetophenone because of the nitro group. Step (5) will be slower for the same reason. The formation of the iodo intermediate may be observed by the formation of a white precipitate before the final formation of yellow iodoform. In the case of hydroxyacetophenone no intermediate step is observed and the first formed precipitate is the yellow iodoform.

Theoretical Considerations

The following equilibria are involved when iodine is placed in aqueous solution. The equilibrium constants are given for 25°C.

$$I_3^- \rightleftharpoons I_2 + I^- \qquad K_{eq} = 1.4 \times 10^{-3}$$

$$HIO \rightleftharpoons H^+ + IO^- \qquad K_{eq} = 1.0 \times 10^{-11}$$

$$I_2 + H_2O \rightleftharpoons H^+ + I^- + HIO \qquad K_{eq} = 3.0 \times 10^{-13}$$

From these equilibria it can be shown that in mixtures of iodine in potassium iodide ($I_2 = 4 \times 10^{-3}\ M$, $I^- = 1.6 \times 10^{-2}\ M$) in alkaline solution, the maximum hypoiodous acid concentration is reached at *p*H of 11.4 and that the hypoiodous acid is converted almost completely to hypoiodite ion at *p*H 13.0 (*5*).

In alkaline solution the hypoiodous acid is unstable, decomposing to iodate and iodide ions, the rate of decomposition being given by the rate equation at 25°C.

$$\frac{-d(IO^-)}{dt} = 2.9(IO^-)^2 + \frac{104(IO^-)^2(I^-)}{(OH^-)}\ \text{mole}^{-1}\,\text{min}^{-1}$$

the reaction having an activation energy of 12,400 calories (*6*).

For acetone the equilibrium with enol at 25°C is (*7*)

$$CH_3COCH_3 \rightleftharpoons CH_3COCH_2^- + H^+ \quad K_{eq} = 10^{-19}$$

and the rate of formation of enol from undissociated acetone is given at 25°C by (*8*).

$$\frac{-d(I_2)}{dt} = 31(OH^-)(CH_3COCH_3)\ \text{mole}^{-1}\,\text{min}^{-1}$$

the reaction having an activation energy of 13,400 calories.

The order of the electrophilic strength of the iodine carrying species is $I_2 > HIO > IO^-$ and the equilibria show that the concentration of the most powerful species (I_2) diminishes with increasing hydroxyl ion concentration. Increase in alkali concentration facilitates reactions (1) and (3).

Except when R contains deactivating groups the concentration of $RCOCH_2^-$ is usually low and does not limit the rate of reaction (2).

Alkaline hypoiodous acid solutions undergo decomposition at room temperature. Comparison of the activation energies shows that the rate of decomposition is enhanced to a greater degree with increased temperature than is the rate of enolization of acetone. The rate of

decomposition of hypoiodous acid increases as the alkali concentration increases up to *p*H 12 due to the formation of hypoiodite ions. The rate then diminishes as the second term in the rate equation becomes more significant.

It has been found from quantitative studies of the iodoform reaction with acetone that because of the decomposition of the hypoiodite it is advisable to have the concentration of alkali about twenty times that of acetone (*1*). It would thus appear that the best condition for the iodoform reaction would be in strong alkali. However, because the general effect of alkali is to facilitate the removal of protons which in the presence of an oxidizing agent will facilitate electron removal, it is advisable to keep the alkali concentration as low as possible. A plausible pattern for the oxidation is presented later.

We conclude on theoretical grounds, therefore, that the optimum condition for the formation of iodoform is at the minimum concentration of hydroxyl ions consistent with the maximum concentration of the strongest electrophile stable at this *p*H. This concentration is established simply by adding sodium hydroxide to iodine in potassium iodide solution to a point where the iodine color just disappears.

Experimental Procedure

A method of testing which incorporates these theoretical principles is given below. It does not differ greatly from many methods described in the literature which appear to have been derived from the original paper of Fuson and Tullock (*4*).

To a film of liquid or a small crystal of solid[a] add 3 ml of an aqueous solution of 0.10 *N* iodine dissolved in the minimum concentration of potassium iodide. Add 2 *N* sodium hydroxide until the iodine color just disappears.[b] A yellow precipitate of characteristic odor indicates the formation of iodoform.[c] If no precipitate forms immediately, allow to stand[d] in the cold, adding further iodine[e] in potassium iodide if necessary until a permanent iodine color persists.

Notes:

(*a*) Even if the substance is insoluble in water the reaction will proceed rapidly since one of the final products is the water soluble sodium salt.

(*b*) The experimental conditions conform to those shown to be desirable from theoretical considerations.

(*c*) Warming is unnecessary since if iodoform is going to form it will do so in the cold.

(*d*) Allowing to stand for a short time is sometimes necessary with compounds of the type X—$CH(OH)CH_3$ requiring oxidation, and also if R is a strongly activated or deactivated aryl group.

(*e*) Addition of further iodine in potassium iodide compensates for any hypoiodite converted to iodate and for any iodine lost by substitution in groups other than CH_3CO—.

The following are some empirical observations made using the above test at 20°C. Substances giving the test are divided into two classes: those giving a *rapid* reaction (iodoform formed instantaneously) and those giving a *slow* reaction (iodoform formed after a delay of thirty seconds or more).

Rapid test. Isopropyl alcohol, acetone, acetophenone, methyl propyl ketone, acetaldehyde, *sec*-butyl alcohol, acetophenone, *p*-aminoacetophenone, resorcinol, methyl isobutyl ketone, *p*-benzoquinone, 2-hydroxy-1-4-naphthaquinone, *p*-hydroxyacetophenone, *m*-hydroxyacetophenone, methyl *n*-amyl ketone, acetonylacetone, benzoylacetone, hydroquinone, 2-phenylethyl methyl ketone.

Slow test. Ethyl alcohol, *sec*-amyl alcohol, ethyl acetoacetate, benzalacetone, 1,4-naphthaquinone, 2,4-dihydroxyacetophenone, *m*-nitroacetophenone.

Inferences from the Reaction

We next consider some of the inferences that can be made from the test. The important point is not the fact that certain compounds will give iodoform when treated with iodine and sodium hydroxide, but the inference which can be made from a positive test. It is obvious that no great significance can be attached to the differences in rate with the hypoiodous test except that a slow reaction indicates that the substance is deactivated in one or more of the theoretically possible ways.

We might from the above results formulate the following rules. If a mono-oxygenated compound containing C, H, and O only contains a carbonyl group, then a *positive* iodoform reaction according to the above test indicates CH_3CO—. If a mono-oxygenated compound containing C, H, and O only, contains a hydroxyl group then a positive iodoform reaction indicates $CH_3CH(OH)$—. The important point in the argument is that the iodoform reaction applied to *any* organic compound is *not* specific for any group, but if we limit the context in which it is applied then a positive iodoform reaction indicates a CH_3CO— grouping.

The inference possible from a negative iodoform reaction is very much weaker, as follows: if a mono-oxygenated compound containing, C, H, O only, contains a carbonyl group, then a *negative* iodoform reaction indicates either that the CH_3CO— group is not present or that the carbonyl group is deactivated in some way either electronically or sterically. The limitation to mono-oxygenated compounds is unsatisfactory if we wish to use the test qualitatively, but it arises from the fact that the reagent is an oxidizing agent as well as an iodinating agent and will attack at oxygen centers other than carbonyls. The next step therefore is to make the reagent more specific by limiting the oxidizing properties while, at the same time, retaining the iodinating properties.

Alternate Method Using Iodine Cyanide

If cyanide ions are added to iodine solutions the color disappears due to the formation of colorless iodine cyanide.

$$I_2 + CN^- \rightleftharpoons ICN + I^-$$

Rothlin (*9*) has suggested alkaline iodine cyanide as the basis of a more selective iodoform test. Experiments show that this reagent is in fact less reactive than hypoiodous acid.

Since the iodine cyanide reagent reacts very much more slowly in those cases where oxidation is required we suggest the following plausible mechanism for the oxidation of $RCH(OH)CH_3$ to $RCOCH_3$.

$$CH_3CH(OH)R + OH^- \underset{\text{fast}}{\rightleftharpoons} CH_3CHOR^- \qquad (6)$$

$$CH_3CHOR^- + I_2 \xrightarrow[\text{slow}]{} CH_3CHOIR + I^- \qquad (7)$$

$$CH_3CHOIR + OH^- \xrightarrow[\text{fast}]{} CH_3COIR^- + H_2O$$

$$\xrightarrow[\text{fast}]{} CH_3COR + I^-$$

Qualitative evidence for this mechanism is as follows: the order of reactivity of substituted alcohols is

$$CH_3{-}\underset{\displaystyle CH_3}{\underset{|}{CHOH}} > CH_3{-}\underset{\displaystyle CH_2CH_3}{\underset{|}{CHOH}} > CH_3{-}\underset{\displaystyle H}{\underset{|}{CHOH}}$$

If reaction (6) was slow and reaction (7) rapid, this order would not be observed. In this oxidation scheme both strong alkali and strong electrophile favor the oxidation reaction. Since iodine cyanide is theoretically a stronger electrophile than hypoiodous acid, and in practice the iodine cyanide is less reactive than hypoiodous acid, we presume therefore that the important electrophile in step (7) is the iodine molecule.

From the equilibria previously given we have

$$I_2 + OH^- \rightleftharpoons HIO + I^- \quad K_{eq} = 30$$

For the comparable cyanide-containing solution at 25°C (*10*).

$$I_2 + CN^- \rightleftharpoons ICN + I^- \quad K_{eq} = 9.3 \times 10^8$$

It is easily seen that the concentration of molecular iodine is very much less in alkaline solutions containing cyanide ions than in alkaline solutions containing no cyanide ions. This is especially true in strongly alkaline solution.

An experimental procedure based upon the above theoretical principles is as follows:

To a film of liquid or one small crystal of solid add 3 ml of an aqueous solution of 0.10 *N* iodine dissolved in the minimum concentration of cyanide. Now add 1 ml of 30% sodium hydroxide solution. A *positive* iodoform test is indicated by the *instantaneous* precipitation of iodoform in the cold (20°C). Results observed when using this procedure are as follows:

Rapid test. Acetone, acetophenone, methyl propyl ketone, acetaldehyde, acetophenone, *p*-aminoacetophenone, methyl isobutyl ketone, *p*-hydroxyacetophenone, *m*-hydroxyacetophenone, methyl *n*-amyl ketone, acetonylacetone, benzoylacetone, 2-phenylethyl methyl ketone.

Slow test. Isopropyl alcohol, ethyl alcohol, *sec*-amyl alcohol, *sec*-butyl alcohol, ethyl acetoacetate, resorcinol, benzalacetone, *p*-benzoquinone, 2-hydroxy-1,4-naphthaquinone, 1,4-naphthaquinone, 2,4-dihydroxyacetophenone, *m*-nitroacetophenone, hydroquinone.

Whereas in the case of hypoiodous acid it was not possible to attach any great significance to the differences in rate, inspection of the list of substances tested by the cyanide method suggests that important inferences can be drawn from the rate data.

All the compounds reacting rapidly with the iodine cyanide reagent contain a CH_3CO- grouping whereas all compounds requiring oxidation before the formation of iodoform fail to react rapidly in the iodine cyanide test. *This procedure thus appears more selective than the conventional one.*

The inference we might now make from a positive test (instantaneous reaction with iodine cyanide) is that if a compound contains C, H, and O only then it contains a CH_3CO- grouping. In this case we have removed some of the restrictions of the hypoiodous reaction.

Conclusion

Many of the reactions of qualitative analysis are capable of giving very useful information. A detailed study has been made of the inference that can be drawn from a positive iodoform reaction. By considering fully the theoretical basis of the test procedure it is possible to deduce a rational modification of the test. We believe the approach we have used in this investigation may usefully be extended to other qualitative organic reactions.

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"The New Elements" Reprints Available

The great demand for copies of the January 1959 issue of the Journal of Chemical Education has exhausted the largest press run this journal has ever ordered. In response to the continuing stream of requests, a reprint of the 43-page symposium has been prepared. The book contains the following:

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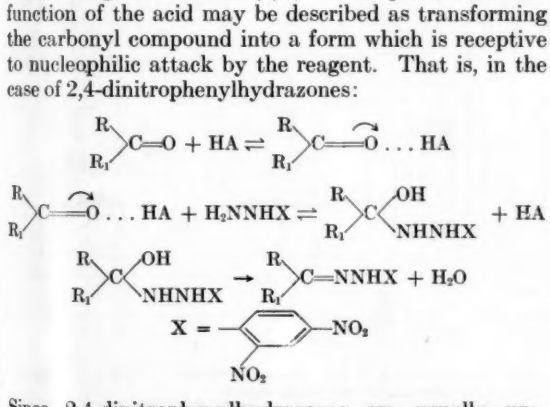
H. J. Shine
Texas Technological College
Lubbock

Preparation of 2,4-dinitrophenylhydrazones:

Demonstrating Acid Catalysis:

The use of 2,4-dinitrophenylhydrazine in identifying aldehydes and ketones is now common in undergraduate organic chemical instruction. While the use of this reagent is described in numerous elementary organic textbooks and laboratory manuals, it is rare to find among these a book which points out the role of acid catalysis in 2,4-dinitrophenylhydrazone formation. Occasionally, an elementary text will note that semicarbazone formation is acid catalyzed without noting, correspondingly, that phenylhydrazone and 2,4-dinitrophenylhydrazone formation are also acid catalyzed. In the writer's experience only one widely used laboratory manual (*1*) specifically discusses catalysis in 2,4-dinitrophenylhydrazone formation, and, further, describes an experiment which illustrates this catalysis. Interestingly, in a recent textbook by the same author (*2*) the impression is given that phenylhydrazone, 2,4-dinitrophenylhydrazone, and semicarbazone formation are, like oxime formation, base catalyzed.

Details of the general acid catalysis in the formation of these derivatives, developed from the work of Conant and Bartlett (*3*), Stempel and Schaffel (*4*), and others, are given clearly in recent books on the mechanisms of organic reactions (*5*, *6*). In simplified terms the function of the acid may be described as transforming the carbonyl compound into a form which is receptive to nucleophilic attack by the reagent. That is, in the case of 2,4-dinitrophenylhydrazones:



Since 2,4-dinitrophenylhydrazones are usually prepared by adding a strongly acid solution of 2,4-dinitrophenylhydrazine to the carbonyl compound (or vice versa), the acid, HA, in this case is very likely $XNHNH_3$; this, though, is not of importance for the present purposes. The fact, however, that it is a common practice to use strongly acid solutions of 2,4-dinitrophenylhydrazine may well be the reason for the general neglect in elementary books and in laboratory manuals of mentioning the mechanistic importance of the acid. That is, the insolubility of 2,4-dinitrophenylhydrazine in suitable common solvents and the need of strongly acid solutions for keeping the 2,4-dinitrophenylhydrazine in solution are stressed, and as a result, the real importance of the acid is overlooked.

The use of solutions of 2,4-dinitrophenylhydrazine in the now readily available dimethyl ether of diethylene glycol, recently reported in preparing 2,4-dinitrophenylhydrazones (*7*), enables acid catalysis to be demonstrated very easily, and also permits a comparison of catalysis between weak and strong acids. The demonstration can be carried out quite easily by students themselves, as follows:

The Demonstration

Into each of two small flasks is pipetted 0.2 ml of benzaldehyde and 5 ml of 2,4-dinitrophenylhydrazine solution (made by dissolving 1 g of the reagent in 25 to 30 ml of diethylene glycol dimethyl ether). No reaction is apparent until an acid is added to each solution. To one flask is added one drop of concentrated hydrochloric acid. The derivative of the aldehyde precipitates immediately. To the other flask is added 5 drops (0.1 ml) of acetic acid. The flask is allowed to stand, and it is observed that the derivative first begins to appear in about 15 minutes, and within a few minutes after that has crystallized out nicely. Thus, the student is able to observe acid catalysis and to obtain also a qualitative idea of the rate of acid catalyzed derivative formation as a function of acid strength.

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Precise Coulometric Titrations Based on Silver Coulometer Measurements

Many methods and kinds of apparatus have been described for carrying out coulometric titrations. The problems involved include the selection of suitable reagents, adequate means for end-point detection, the precise determination of the quantity of electricity involved, and the proper design of apparatus. The apparatus should provide for the separation of the anode and cathode compartments, be as simple as is consistent with the attainment of satisfactory results, and, in cases where gases are evolved, be constructed so that the gases cause little change in electrical resistance. In certain types of apparatus, the evolution of gases can be annoying and in some cases silver anodes in chloride solutions have been used to solve the problem. Indicators or electrometric methods are customarily used for end-point detection. Devices insuring constant current, used with suitable meters and timing apparatus, are frequently employed for the measurement of the number of coulombs required for the titration. In case the current is not constant, readings may be taken at suitable time intervals and a summation made for the titration. The introduction of silver coulometers directly into the circuit as described by L. Szebelledy and Z. Somogyi[2] lessens the importance of constant current and allows the measurements to be made directly in terms of deposited silver. This, of course, requires weight determinations. The purpose of this article is to describe a method for coulometric titrations using a different type of silver coulometer and a versatile type of apparatus with which gas evolution causes no trouble. The combination may be used for precise results. A diagram of a simpler apparatus suitable for a laboratory experiment in coulometric titrations is also shown.

Apparatus and Procedure

Several types of apparatus were developed and used, but the most versatile type is shown in Figure 1. The side arms of the stopcocks A and A' were cut off as close to their shells as possible and the stopcocks sealed to 10-mm tubing which formed a bridge between the anode and the cathode compartments. The stopcock plugs were roughened with abrasive until the resistance of the whole apparatus was about 3000 ohms with the stopcocks wet with electrolyte and closed, and about 1200 ohms with the stopcocks open. The electrodes B and B' were fitted with glass protective shields designed so as to allow the liquid free access to the platinum discs. The electrolyte used was prepared by dissolving 25 g of anhydrous C.P. sodium sulfate in 100 ml of distilled water and adjusting the pH to 7.0.

The method provides for the inclusion of the silver coulometers C and C' in the circuit. This eliminates the necessity of an ammeter and timing device and makes constant current control of little importance; the measurements are made directly in terms of grams of silver deposited. Fifteen per cent $AgNO_3$ solution prepared from the A.R. reagent, with no further purification, was used as the electrolyte in the silver coulometers.

Forty-five-volt B batteries were used as the source of

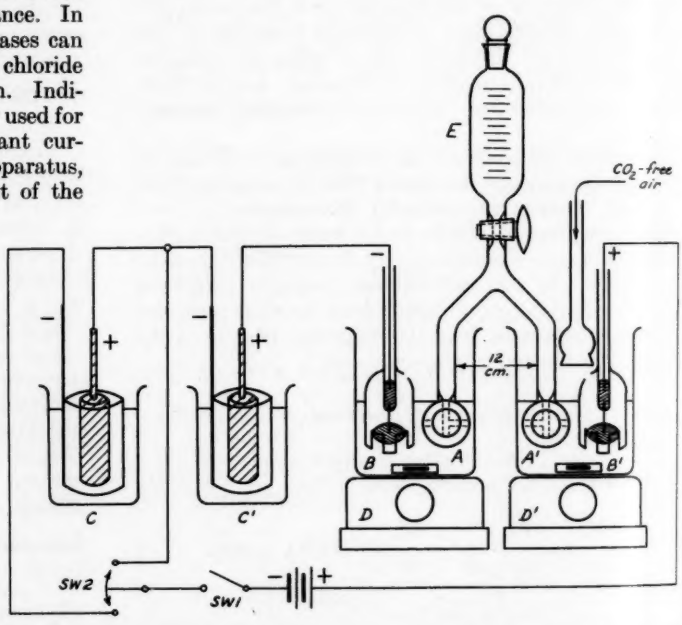


Figure 1. Apparatus for simultaneous coulometric titrations of acid and base, using silver coulometer measurements.

Titration cells, in 250-ml beakers: *A-A'*, 3-way, 1-mm bore stopcocks; *B-B'*, platinum disc electrodes, 17-mm diameter; *D-D'*, magnetic stirrers; *E*, cylindrical graduated separatory funnel, 125-ml.

Silver coulometers, C-C', in 100-ml beakers: Cathodes, platinum foil, 25 × 50 mm; anodes, electrolytic silver rod, 12 × 55 mm, attached to 3.5-mm silver wire; *SW 1*, single-pole toggle switch; *SW 2*, 3-way switch.

[1] Present Address: Department of Chemistry, University of Michigan, Ann Arbor, Michigan.

[2] Szebelledy, L., and Somogyi, Z., "Coulometric Analysis as a Precision Method I," *Z. anal. Chem.*, 112, 313–23 (1938).

current and, with the apparatus described, no additional resistors were required. In actual operation, a milliammeter, Weston Model 1, certified to 0.25% of full-scale reading, was placed in the circuit and current readings recorded every minute throughout the course of the titrations. The cumulative values so obtained were used for purposes of comparison. The currents ranged from 30 to 50 milliamperes, with corresponding times of about 55 to 35 minutes per titration, depending on how the liquid junctions were made. During a given titration, the current varied through a range of 2 to 3 milliamperes.

When the apparatus was used with the stopcocks closed, 90 volts produced a current of about 25 to 30 milliamperes, depending on how tightly the stopcocks were seated. With the stopcocks in the open positions, 45 volts produced a current of about 35 milliamperes. Both methods produced good results; closed stopcocks required less of the Na_2SO_4 solution, while open stopcocks resulted in smaller cell resistance. With the currents used, the evolution of gases at the electrodes was not a problem.

Tests showed that there was no measurable change in the *p*H of the sodium sulfate solution in the bridge during a titration. The apparatus was used with the stopcocks open in two directions and open only in one direction as shown in Figure 1. The latter position was preferred because it permitted essentially no gas to enter the bridge. The rate of flow of about 30 drops per minute was controlled by the adjustment of the stopcock on *E* and could be checked by way of the graduations on the dropping funnel. About 125 ml of Na_2SO_4 solution were used during the titration of approximately 1 milliequivalent of acid or base.

In the course of this investigation, the simple apparatus shown in Figure 2 was also developed. Used for titrations of a few tenths of a milliequivalent, it has proved convenient for laboratory instruction.

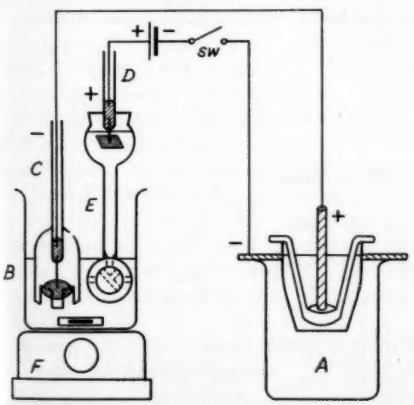


Figure 2. Simplified coulometric titration apparatus for laboratory instruction.

Silver coulometer (Kohlrausch-type), *A*: Cathode, 15-ml platinum crucible in contact with copper wire triangle supported on a 250-ml beaker; anode, silver wire or strip; anode cup, glass cup fused to glass rod hangers; electrolyte, 15% $AgNO_3$.

Titration cell, *B*, in 250-ml beaker: C, platinum disc electrode, 17 mm diameter; *D*, platinum foil electrode, 1 cm^2; *E*, thistle tube-stopcock bridge filled with Na_2SO_4 electrolyte; *F*, magnetic stirrer.

Simultaneous standardizations of an acid and a base are possible with the apparatus shown in Figure 1, and such determinations were made in order to test the accuracy and precision of the method.

At the beginning of the titration, the solution in the cathode compartment consisted of 75 ml of the Na_2SO_4 solution, 10.00 ml of 0.1000 *N* potassium acid phthalate solution (prepared from the NBS reagent), and 3 drops of 0.1% phenolphthalein indicator in ethanol. The solution in the anode compartment consisted of 60 ml of the electrolyte, 25.0 ml of saturated $Ca(OH)_2$ solution, and 3 drops of the phenolphthalein indicator. The saturated $Ca(OH)_2$ solution was prepared from $Ca(OH)_2$ that had been washed thoroughly with three portions of CO_2-free distilled water. In storage and in use the $Ca(OH)_2$ solution was protected from the action of CO_2 in the air. When flowing junctions were used, care was taken to equalize the levels of the liquids in the two beakers before the latter were placed in position under the stopcocks in order to prevent either the acid or the base from flowing into the bridge arms at the beginning of the titration. The flow of Na_2SO_4 solution was started just prior to the placing of the beakers with their solutions under the stopcocks and an atmosphere of CO_2-free air was maintained in the anode compartment. Although phenolphthalein was used as indicator in both compartments, the end points were checked occasionally with a *p*H meter. The end points in the two compartments did not occur at the same time and a switching arrangement was used so that one silver coulometer measured the neutralization of the base at the anode while the other measured the neutralization of the acid at the cathode.

The general specifications of Rosa and Vinal of the National Bureau of Standards[3] were modified where necessary and applied to the silver coulometers shown in Figure 1. Initially, the cylindrical platinum foil cathodes were washed by dipping and rinsing them in water and drying them with acetone and air. This procedure proved unsatisfactory due to mechanical loss of silver. The following procedure was, therefore, developed and it proved satisfactory.

The cleaned platinum foil cathodes in their respective 100-ml beakers were dried, cooled, and weighed as units; a similar 100-ml beaker was used as a tare. The cathodes and anodes were suspended from their supports and other 100-ml beakers were used to contain the $AgNO_3$ solution during the titration. After the titration was completed, the anodes and the beakers containing the $AgNO_3$ solution were removed. Any anode slime or particles of silver mechanically dropped from the anode remained in these beakers and were thus removed. The cathodes were carefully positioned in their respective, previously weighed beakers. There was no evidence that any electrolytically deposited silver was lost from the cathodes during these operations. The cathodes were then washed with 300 ml of distilled water. The wash water was removed from the beakers by suction through a medium coarse filter stick extending to the bottom of the beaker, and any particles of silver dislodged from the cathodes by washing were thus retained in the beakers. Usually no dislodged silver was discernible, but when such particles were present, careful procedure left them adhering to the bottom of the beaker. Any particles

[3] Rosa, E. B., and Vinal, G. W., "Summary of Experiments on the Silver Voltammeter at the Bureau of Standards and Proposed Specifications," Scientific Papers of the Bureau of Standards, No. 285, Oct. 5, 1916.

Table 1. Comparison of Silver Coulometer with Time-Meter Results for Simultaneous Coulometric Titrations of Acid and Base

Run No.	Anode (base) [a] Taken: 113.0 mg Ag				Cathode (acid) [b] Taken: 107.9 mg Ag			
	Coulometer		Time-meter		Coulometer		Time-meter	
	Found, mg	Diff., mg	Found, mg	Diff., mg	Found, mg	Diff., mg	Found, mg	Diff., mg
1	113.0	0.0	112.9	−0.1	107.9	0.0	107.6	−0.3
2	112.9	−0.1	112.3	−0.7	107.9	0.0	107.5	−0.4
3	113.0	0.0	113.1	+0.1	107.9	0.0	107.8	−0.1
4	112.9	−0.1	112.7	−0.3	107.9	0.0	107.8	−0.1
5	112.9	−0.1	112.9	−0.1	108.0	+0.1	108.0	+0.1
6	113.0	0.0	112.7	−0.3	107.8	−0.1	107.7	−0.2
7	113.2	+0.2	113.2	+0.2	107.9	0.0	108.0	+0.1
8	113.0	0.0	113.0	0.0	107.9	0.0	108.0	+0.1

[a] Average of the eight coulometer results shown.
[b] Theoretical values calculated from the volume of standard acid solution taken for the titration.

adhering to the filter stick were washed into the beaker. The beakers and cathodes were dried at 110°C for an hour, cooled, and weighed.

Typical Results

The results of a series of simultaneous titrations of acid and base, which attest to the precision and accuracy of the method and apparatus, are shown in Table 1. The results of runs 1 through 5 were obtained with flowing junctions while those of runs 6 through 8 were obtained with the stopcocks closed.

The data show that the reproducibility was slightly better in the cathode compartment than in the anode compartment and this probably was due to the difference in the end points. This involved the detection of color appearance in the cathode compartment and color disappearance in the anode compartment. The average deviation calculated for the anode compartment was ±0.1 mg of silver based on the average value of 113.0 mg for the eight determinations shown in Table 1. The normality of the saturated $Ca(OH)_2$ solution, calculated on the basis of the weight of silver deposited was 0.04189 ± 0.00001.

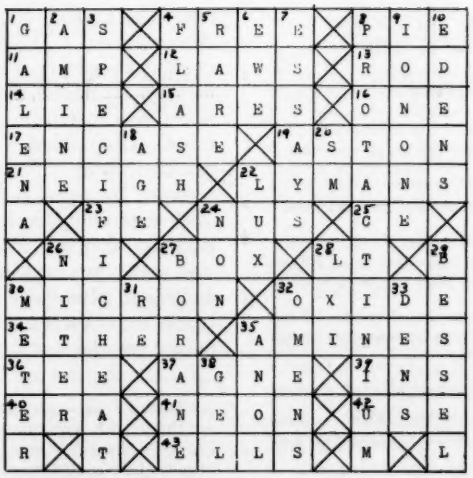
Many more determinations were made on the acid than on the base and in another series of 12 titrations of the acid, exclusive of those in Table 1, the average weight of silver deposited (calculated to 0.01 mg) was 107.88 mg with an average deviation of ±0.06 mg. Strict adherence to quantitative techniques is essential and any deviation therefrom will affect the results adversely. The accuracy and precision of the apparatus and method probably could be increased tenfold if enough time were allowed for the deposition of about 1 gram of silver. This would require, however, about 6 or 7 hours with currents of about 30 to 50 milliamperes. With this apparatus, currents of about 55 milliamperes and above were inadvisable because gases were evolved so rapidly around the electrodes with such currents that the resistance of the system fluctuated widely. The ultimate precision of this method probably approaches that of the silver coulometer but is dependent on end-point detection.

This method and type of apparatus are suitable for titrations other than those of acidimetry, and it is planned to investigate some of these in the future.

Acknowledgment

The authors wish to express their appreciation to the Graduate Council of Southern Illinois University for a grant which helped support this investigation.

Answer: Crossword Puzzle (page 529, October)



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Rapid Dispensing of Liquids for Large Laboratory Classes

When solutions or liquid reagents are made available to students in stock bottles, there is a marked tendency for the students to spill some of the liquids and take somewhat more than is needed. In the case of corrosive liquids, the spillage becomes an even greater nuisance. The spillage and wastage can be reduced greatly by offering the liquids in burets or pipets of the automatically filling type, but such dispensing is slow and many of these fragile glass dispensers are required for large laboratory classes.

We have found that this problem can be handled conveniently and at reasonable cost by the use of automatic syringes. These devices, which are available from most laboratory supply houses, are ordinarily used for highly repetitive dispensing problems, such as loading test tubes with media for bacterial assays, loading vials with pharmaceutical solutions, or adding reagents for analysis of many samples. They are essentially small pumps which deliver a constant volume with each stroke. The automatic syringes may be obtained in motor-driven types and hand-operated types.

Some of the basic elements of the automatic syringe are shown in the accompanying figure. A hypodermic syringe (*A*) serves as the pump. The metal part (*B*), which attaches to the tip of the syringe, contains two valves. The inlet valve, on the side, leads by a flexible tube (*C*) to the stock bottle containing the solution to be dispensed. The exit valve, on the bottom, leads by a wide bore needle (with a blunt end) (*D*) to the student's test tube or flask. The syringe is held in a metal device which contains a spring and a plunger. When the student presses down on the plunger, the glass syringe plunger is forced down and liquid is expelled into the container. Release of the plunger allows the spring to return the glass plunger and refill the syringe from the stock bottle. A screw adjustment (*E*) in the metal holder controls the amount of liquid dispensed with each stroke.

We have been using two commercially available automatic syringes, the Adams Aupette[1] and the Cornwall continuous pipettor.[2] In the case of the former, we recommend only the 5-ml size; the 1-ml size is too fragile for student use and the 10-ml size seems to have a spring that is too stiff for girls. In the case of the latter device, we recommend the 2-ml and the 5-ml sizes.

The metal valves make the apparatus unsuitable for use with acidic solutions. Two days' exposure to 0.2 *N* NaOH is not detrimental. Use with organic solvents requires polyethylene or Teflon tubing.

When strong acids are to be dispensed, we replace the metal valves with Teflon valves.[3] These valves have delivery tips which do not require needles. We use polyethylene tubing[4] between the inlet valve and stock bottle. Because of a difference in dimensions, only the Aupette syringe holder can be used with the Teflon valve; it is necessary to replace the Aupette syringe by an ordinary 5-ml hypodermic syringe with glass tip.

Certain techniques in the use of these syringes are helpful. First, it is usually necessary to keep the level of liquid in the stock bottle below the tip of the automatic syringe to avoid siphoning. Second, it is necessary to prime the pumping system each time a new solution is started. Third, it is useful to hang a card over each automatic syringe advising the student as to the number of "squirts" to take and which container to use. The syringes tend to trap a small air bubble (from dissolved air), and the valves do not operate precisely the same way with each stroke, so the reproducibility of delivery volumes is not as good as that of a volumetric pipet. For this reason, the syringes are not used to dispense exact portions of standards or unknowns for quantitative analysis.

While we have used this technique only in the biochemistry laboratory, it may be found useful in other student laboratories. Although the technique cannot be economically extended to the dispensing of large volumes, one can sometimes use concentrated solutions and have the students dilute them at their tables.

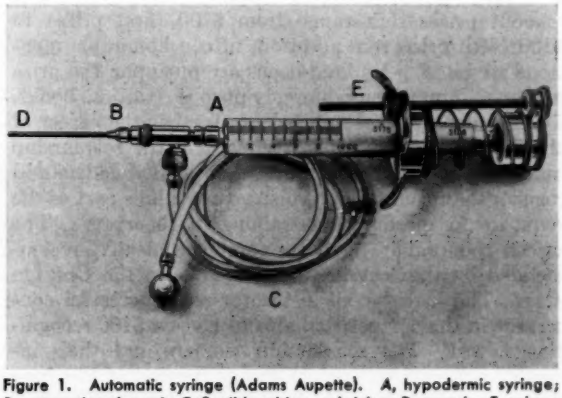

Figure 1. Automatic syringe (Adams Aupette). *A*, hypodermic syringe; *B*, automatic valve unit; *C*, flexible tubing and sinker; *D*, cannula; *E*, volume control. (Photograph by courtesy of Scientific Products, Evanston, Illinois.)

[1] Clay-Adams Co., No. A-2700.
[2] Becton, Dickinson and Co., No. 1251 (2 ml) and No. 1261 (5 ml). Furnished with cannula No. 1250 NR.
[3] Tri-R Instruments, 144-13 Jamaica Ave., Jamaica 35, N. Y.
[4] Clay-Adams Co.

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Chicago Section ACS Scholarship Examinations for High School Students

Each spring, for the past four years, the Chicago Section has fostered a competitive examination in chemistry for Chicago area high school students who are particularly talented in this field of science. The Chicago area includes the city and suburban high schools as well as the private schools located in Chicago and its suburbs.

The objectives of the Chicago Section are to encourage youth to develop their aptitudes in the field of chemistry and to enhance the importance of the instructor of chemistry on the secondary school level. The success encountered by this section may induce other sections to adopt a similar program.

The annual Scholarship Examination carries with it monetary prizes totalling $2,200. There are five student prizes that range from $700 (first prize) to $100 (fifth prize); in addition, fifteen honorable mentions are cited. No limitations are put upon the prize money as it may be used for any purpose (tuition, books, living expenses) that will help to further the career of the young scientist. The recipient of the scholarship must, however, enroll in an ACS accredited college and register for a course in chemistry that fulfills part of the requisite for professional training in chemistry. The fifteen honorable mentions are specifically recommended by the section to universities in the Chicago area. The high school instructor of a successful contestant in the competition also receives a $100 recognition award. The scholarship winners and their instructors are guests of the Chicago Section at the June dinner meeting where the awards are presented.

The Scholarship Subcommittee prepares and administers the examination. For the first three years the examinations were only in the form of objective tests. The total score for each test was 1000 points. The examination for 1958 was composed of an objective test with 700 points and a subjective test with 300 points. The latter was incorporated to determine the examinee's ability to present technical information coherently and concisely. The examination is given in the forenoon on the second Saturday in May in the chemistry auditorium of the university which is host to the examinees.

The selection of the examinees is the responsibility of the high school instructors of chemistry and is based on the following procedure. Early in the fall semester cards, announcing the tentative date of the examination and inviting the students who excel in chemistry to participate in the contest, are mailed to the chemistry instructors. In doing this, sufficient time is afforded to the students to compete among themselves for participation in the contest and to the instructors to observe the performances of their students. Each instructor may choose *two* of his most capable students for the competitive examination. Cards for the registration of the elected contestants are mailed to the respective high schools early in the spring semester. The cards also state the time, date, and place where the examination is to be conducted. On the basis of the number of registrants so obtained, the host university plans a gratuitous luncheon.

Since the inception of this program, the number of high schools represented in the contest has increased from 90 to 111. This increase is primarily due to the greater number of private schools which now participate. The number of contestants from suburban, urban, and private schools for 1956 to 1958 are listed in Table I. (The incomplete figures for 1955 are not included.) It is hoped that every high school within the Chicago area will soon participate in this program.

Table 1. Number of Contestants for 1956–58

Year	Suburban schools	Chicago schools	Private schools
1956	91	65	40
1957	97	63	60
1958	97	64	61

To create an appropriate picture of the effects which may be attributed to this annual competitive examination, the results of the objective tests from 1955 to 1958 will be summarized first, and subsequent to this the results of the single subjective test will be reviewed briefly.

Since the same examination is administered to three different groups of high schools, it is not difficult to imagine that this, in itself, serves as an incentive for each group to project itself most favorably. Although to date the students from the suburban schools still predominate on the prize winning and honorable mentions lists, the students from the Chicago schools have moved upward most notably on both lists within the last two years. Furthermore, the number of students from private schools on the honorable mentions list has increased considerably within the same period of time, and this increase is accompanied by a pronounced decrease in the lowest percentile. These facts suggest that the annual Scholarship Examination motivates inspirational teaching and, on the part of the student, increased intelligent thinking. The latter surpasses the former in influencing the performance of the examinee. This is evidenced by the observation that the test scores of two students having the same instructor may vary considerably.

All the objective tests are analyzed statistically.

This not only determines the adequacy of the items comprising the tests but also permits an evaluation of the progress of the contestants from year to year. In general, it is observed that the lower half of the examinees is more familiar with the history of chemistry than the upper half. In contrast, the upper group surpasses the lower in the application of the principles of elementary physical chemistry. This indicates that the performance of the lower group is conditioned by the capacity to memorize, and that of the upper group by the ability to reason inferentially. (Inferential reasoning, on part of the examinees, was rather limited in 1956 since only two per cent of the examinees answered 40% of the multiple choice questions correctly. In contrast to this 15% of the examinees answered all the multiple choice questions correctly in the 1958 examination.) This remarkable upswing is all the more notable, since the 1958 examination is classed as the more difficult of the two tests. Multiple choice questions challenge the examinee's ability to discriminate and to draw correct conclusions. Such ability can only be developed by good pedagogy. This substantiates the previous claim that the annual Scholarship Examination motivates inspirational teaching.

The performance of the contestants in the subjective test is, at the present time, only in fair alignment with their performance in the objective test. A high score in the objective test may or may not be accompanied by a coherent discussion of a selected scientific topic. Thus the Scholarship Examination also makes evident the great need for training the students in expressing their thoughts in writing.

Finally, it is pointed out that every one of the prize winners has an excellent rating at the university of his choice, and some have already received new honors. This fact alone is adequate assurance that the policy of sponsoring the annual Scholarship Examination is a judicious investment.

The Chicago Section of the American Chemical Society plans to continue the sponsorship of the competitive examination for high school students in the Chicago area as a means of encouraging and rewarding better teaching and student achievement in chemistry. The fact that only outstanding students in chemistry are nominated for the examination serves, in itself, as a recognition of the superiority of all examinees. It is hoped they will be further encouraged to develop their potentialities into actualities. Details of the program are available from the authors upon request.

[1] Members of the Education Committee of the Chicago Section of the American Chemical Society.

S. Aronoff
Iowa State College
Ames

Code for Alphabetical Index for Punched Cards

Punched cards for indexing[1] have evolved along three major lines: (1) numerical (letters are given the number 01 →26, corresponding to their alphabetical position); (2) OIECB (letters are given a code involving one or more of these five letters); and (3) the triangular code (letters are arranged in a triangle and may be read directly, according to a designated coding system). The first system requires four pairs of holes; the second, five; and the third, six. The first and second systems require extensive memorization. Occasionally the latter requires triple or quadruple punches, while the triangular system requires additional space both horizontally and vertically. The numerical system provides no direct-reading letters, while the OIECB provides the five.

The following code is adapted readily from numerical code cards, requiring only four pairs of holes. It provides eight direct-reading letters with the residual letters being interpolated readily. No more than two punches are required for any letter. It is therefore as economical as the numerical system, but simpler than either the numerical or OIECB and requires less space then the triangular.

•	•	•	•
J	H	E	A
•	•	•	•
W	S	O	K

The letters indicated are direct-reading. The intermediate letters are formed by one additional punch in sequence from right to left. Letters prior to *K* require two *short* punches; those subsequent require one long punch and one short one. The following code is thus obtained:

A = A	N = KJ
B = AE	O = O
C = AH	P = OA
D = AJ	Q = OH
E = E	R = OJ
F = EH	S = S
G = EJ	T = SA
H = H	U = SE
I = HJ	V = SJ
J = J	W = W
K = K	X = WA
L = KE	Y = WE
M = KH	Z = WH

[1] Casey, R. S., et al., *Editors*, "Punched Cards," 2nd ed., Reinhold Publishing Corp., New York, 1958, pp. 18–22.

Proceedings of the

Pacific Southwest Association of Chemistry Teachers

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Papain as an Enzyme Catalyst in Undergraduate Organic Chemistry

Papain has become a well-known commodity on the American market in recent years because of its meat tenderizing activity. To the chemist, this is known to be a consequence of the ability of this enzyme to catalyze the hydrolysis of special types of amide linkages, particularly those involved in polypeptides of protein molecules. Also, papain catalyzes the hydrolysis of other peptide-like bonds and esters of amido acids. The origin of the enzyme is the papaya fruit, grown in Ceylon, Burma, South Africa, and America in states such as Hawaii and Florida. Until recently, Ceylon was the principal source of the enzyme, from the juice of the Carica-papaya. At present, the best papain is obtained from papayas grown in South Africa.

In the preparation of commercial and more highly purified crystalline papain, the latex from unripe papayas is first collected while the fruit is still on the tree by making gashes in the fruit and allowing the latex to flow into appropriate receptacles. It is then dried. The resultant solid is treated according to various intermediate procedures. After this, it is often ground with water and sand in a mortar, filtered, and fractionally crystallized with the aid of ammonium sulfate and sodium chloride. Crystals are allowed to dry (*1*, *2*) and are suitable for activation.

Although most enzymes are not at all adaptable to undergraduate laboratory work in organic chemistry, papain is an outstanding exception. It is readily available, easily activated, inexpensive, and conveniently stored by refrigeration for long periods of time. It catalyzes many reactions at a rate convenient for undergraduate observational work. These reactions can be selected to take place between reactants that are inexpensive and available in quantity. Products can be obtained in good yield, purified by simple means, and identified by melting point determinations. If the melting points are high, a melting point bath of 60% concentrated sulfuric acid and 40% potassium sulfate (*3*) gives students experience in surmounting the difficulty of bath decomposition. This mixture is good for temperatures above 365°C. A Fisher-Johns melting point block is often unavailable to undergraduate students, but is usually satisfactory for most temperatures encountered.

The use of papain in catalyzing the formation of peptide-like linkages, rather than their hydrolysis, was first demonstrated by Max Bergmann and Heinz Fraenkel-Conrat in 1937 in a series of reactions between amido acids and aniline or phenylhydrazine (*4*). Since that time, a large number of related investigations has followed (*5*, *6*, *7*). Hippuric acid (benzoylglycine) was shown to give a substantial yield of either the anilide or the phenylhydrazide.

$$\underset{\text{(Hippuric Acid)}}{C_6H_5CNHCH_2COH} + \underset{\text{(Aniline)}}{H_2NC_6H_5} \xrightarrow[p\text{H} \cong 4.5\text{–}5.0]{\text{Papain, 40°C.}} C_6H_5CNHCH_2CNHC_6H_5 + H_2O$$

(Hippuric Anilide)

and

$$\underset{\text{(Hippuric Acid)}}{C_6H_5CNHCH_2COH} + \underset{\text{(Phenylhydrazine)}}{H_2NNHC_6H_5} \xrightarrow[p\text{H} \cong 4.5\text{–}5.0]{\text{Papain, 40°C.}} C_6H_5CNHCH_2CNHNHC_6H_5 + H_2O$$

(Hippuric Phenylhydrazide)

The insolubility of the product is the chief driving force of this reaction, which is actually reversible. It has been demonstrated that separations of racemic mixtures of amido acids can be accomplished with ease (*4*). Carbobenzoxy-DL-leucine yielded primarily the anilide of the L-isomer as an insoluble product while the D-isomer remained unreacted upon in the solution as a consequence of the configuration of papain and therefore its specificity.

$$\underset{\text{(Carbobenzoxy-DL-leucine)}}{C_6H_5CH_2OCONHCHCOOH}\ \ \underset{(CH_3)_2CHCH_2}{|} + H_2NC_6H_5 \xrightarrow[p\text{H} \cong 4.5\text{–}5.0]{\text{Papain, 40°C}} C_6H_5CH_2OCONHCHCONHC_6H_5 + H_2O$$

$$\underset{(CH_3)_2CHCH_2}{|}$$

(Anilide of Carbobenzoxy-L-leucine)

Carbobenzoxy-DL-leucine is rather insoluble itself,

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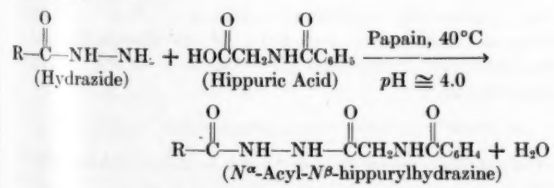
[2] Fresno County Heart Association undergraduate fellow, 1957–58; winner of California Heart Association undergraduate research award, 1957.

so that in general practice other amido acids are sometimes preferably employed as substrates. The specificity of the enzyme was explained by Max Bergmann and Joseph S. Fruton (*8*) to result from a three-point contact between the amido acid substrate and the enzyme surface. The mechanism is largely explained as a contact with an energy-rich thiolactone structure at the active site of papain (*9*, *10*) and is in accord with the general principles of enzyme action (*11*, *12*).

Organic Laboratory Experiments

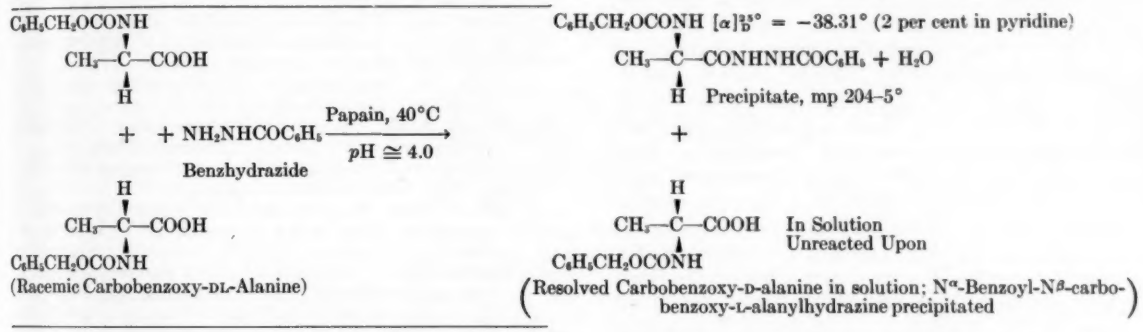
A rather large number of papain-catalyzed reactions can be selected for use in undergraduate organic laboratory work. Variations of the basic, amino-containing reactant involve less expensive reagents. Most of the amido acids are quite expensive, thus imposing rather drastic restrictions in their usage. The behavior of hippuric acid toward aniline (*4*), *p*-aminophenol (*13*), pehnylhydrazine (*4*), *p*-anisidine (*13*), *m*-aminoacetophenone (*13*), and other substituted anilines (*13*) provides a series of substrate combinations of easy application and moderate cost. The melting points of the products are: hippuric anilide, mp 212.5°; hippuric phenylhydrazide, mp 184.5°; hippuric *p*-hydroxyanilide, mp 243–4°; hippuric *p*-methoxyanilide, mp 217–18°; hippuric *m*-acetylanilide, mp 229–30°.

Another such series of combinations would be the reactions between amido acids like hippuric acid and benzhydrazide or other hydrazides (*10*) to yield N^{α}, N^{β}-diacylhydrazines.



Nomenclature is a bit tedious, but with patience the students soon manage this with ease. These reactions proceed very rapidly and are often largely completed within 12 hours. The optimum *p*H is about 4.0, in contrast with a *p*H of 5.0 for anilide formation. This has an added feature of involving an easy synthesis of a hydrazide from hydrazine and methyl or ethyl benzoate (*14*). With hydrazine playing such an important role in modern chemistry, it is available in quantity for such work. The usual precautions must be shown in its usage. Papain-catalyzed reactions with hydrazides point up the difference between the acidity of an acylated amino group and the basicity of an amino group not joined to an acyl radical, both of which occur in benzhydrazide.

Replacement of hippuric acid with carbobenzoxy-DL-alanine provides a means for a demonstrable resolution of a racemic mixture. Carbobenzoxy-DL-alanine (mp 114–15°) is commercially available[3] but is somewhat costly. Directions are given for the preparation of carbobenzoxy-DL-alanine (*15*) from benzyl chloroformate, which is sold commercially under the name of carbobenzoxy chloride.[4] One person in the class can substitute carbobenzoxy-DL-alanine for hippuric acid. The entire class can observe the negative rotation of the resultant N^{α}-benzoyl-N^{β}-carbobenzoxy-L-alanylhydrazine in a pyridine solution.



Activation of the enzyme by means of hydrogen sulfide is carried out by a very simple modification (*13*) of the original method of Grassmann (*16*). Papain is dissolved in ice-cold water and the solution is placed in an ice bath while hydrogen sulfide is passed through the solution overnight. Precipitation of the enzyme with methanol or ethanol is followed by centrifugation at 2000 rpm. The enzyme can then be used directly as a paste, without bothering to dry it. It must be stored under refrigeration to prevent molding. If prepared considerably beforehand, the paste can be dried one week over phosphorus pentoxide. It is usually necessary to decant the alcohol which separates during the drying operation at the end of each of the first two days. An amber or light tan, brittle, solid results which is crushed very lightly and stored in stoppered vials that are kept in a brown bottle with a screw cap in a refrigerator. In our experience we have found that activated papain stored in this way has retained a very high degree of activity even after two years of storage.

Two excellent sources of unactivated, dried, papain latex are the Wallerstein Laboratories[5] and the Schwarz Laboratories.[6] It should be emphasized that papain

[3] K and K Laboratories, Inc., 177–10 93rd Avenue, Jamaica 33, N. Y.

[4] Mann Research Laboratories, Inc., 136 Liberty Street, New York 6, N. Y.

[5] Wallerstein Company, Inc., 180 Madison Avenue, New York 16, N. Y.

[6] Schwarz Laboratories, Inc., 230 Washington Street, Mount Vernon, N. Y.

[7] The senior author (J. L. A.) is indebted to Dr. Heinz Fraenkel-Conrat of the Virus Laboratory, University of California, Berkeley 4, California, for this warning through private communication.

from some sources[7] cannot be satisfactorily activated for this work. A small amount of L-cysteine hydrochloride is used as a promotor for these reactions. Temperature control around 38–40°C should be possible in a crudely devised incubator consisting of an electric light bulb and appropriate packing in a box, in the event an incubator is not available. The enzyme should not be heated above 50°C, and even keeping it below 45°C is probably wise, during any of the procedures involving its preparation and use. This avoids any possibility of deactivation. Solutions can be buffered at *p*H 4.5 to 5.0 with the use of sodium acetate and acetic acid and at *p*H 4.0 by means of sodium lactate and lactic acid. The total salt and acid concentration used as the buffer should be about 0.5 *M*. Partial neutralization of lactic acid is a convenient way of getting this latter buffer mixture.

Acknowledgment

Research related to this paper was supported by the Sigma Xi Society and RESA, the Research Corporation, the Fresno County Heart Association, and the California Heart Association. Dr. Kendall Holmes, Dr. Robert D. Beech, and Mrs. Joyce Richardson of the Fresno County Heart Association, and Dr. John J. Sampson, Dr. Robert Maybury, and Miss Phyllis Hecker of the California Heart Association were particularly instrumental in securing these grants. Students in the undergraduate organic laboratories practiced on some of these reactions. Vice Chancellor William G. Young of the University of California, Los Angeles, suggested avenues of approach for securing research funds. Professor Charles D. Hurd of Northwestern University and Professor Carl Niemann of the California Institute of Technology gave certain advice in connection with the work. Professor Ennis B. Womack of Fresno State College made available certain equipment. Assistance was also given by Mr. Calvin Johnson, Mr. Dennis Karle, Mr. Ronald Johnson, and Mr. Rodney Johnson.

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ChemEd

LETTERS

To the Editor:

The molality-molarity paradox (Toby, J. Chem. Educ., **36**, 230 (1959)) is artificial rather than apparent. From the definitions of the two quantities it is quite obvious that they may be equal for solutions of density greater than one, but cannot be so for solutions of unit, or lower, density.

If density is greater than one, then one l of solution corresponds to more than one kg of solution and M may or may not equal m. If, on the other hand, density is less than one, then one l of solution corresponds to less than a kg of solution (and therefore of solvent) and M cannot equal m.

By setting the dimensionally incongruous definitions of molality and molarity equal,

$$m = 1000\ w/pW = M = 1000\ wd/p(W + w)$$

one obtains the illegitimate result $w = W(d - 1)$ and thus an illegitimate paradox. If, however, molality and molarity are equated ($M = m = x$) in the identity relating them

$$M = 1000\ md/(1000 + mp), \text{ then } d = (xp + 1000)/1000$$

showing that when m and M are equal, d must exceed one.

Arthur C. Hall

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Erratum

Frederick J. Guerin, Lawrence, Massachusetts, advises us that Professor A. E. Hill was associated with New York University, not Yale University, as given in the tabulation on page 440 of the September 1959 Journal of Chemical Education.

Chem Ed

BOOK REVIEWS

The Fundamentals of College Chemistry

G. Brooks King, The State College of Washington, and *William E. Caldwell*, Oregon State College. 3rd ed. American Book Company, New York, 1959. vii + 592 pp. 14.5 × 22 cm. $6.25.

The text has been reworked with a resulting gain in ease of understanding. There are few changes of a drastic nature and very little of the material of the previous edition has been omitted. Among the sections added or improved by rewriting are those on the activity series, effusion and diffusion, physical and chemical atomic weights, oxidation numbers, balancing oxidation-reduction equations, the products of oxidation and reduction, metal hydrides, detergents, and the naming of the higher hydrocarbons. The chapter formerly titled Chemical Equilibrium becomes Reaction Rates and Chemical Equilibrium and the chapter on Radioactivity and Nuclear Changes has been revised and now includes material on the fundamental particles of matter, mass-energy relationships, nuclear power plants, binding energy, the hydrogen bomb, peacetime uses of atomic energy, and radioactive dating. An appendix has been added on oxidation-reduction potentials.

A few exercises have been dropped, but replaced with new ones. Some of the references have been deleted, but replaced by twice as many new, and more recent, references. The illustrations have been redrawn. The diagrammatic line drawings of the second edition are supplanted by perspective drawings done on a background of grey wash. The juxtaposition of greys and whites is more interesting artistically, the use of perspective often gives a truer representation of the apparatus illustrated, but frequently the older diagrams had a clarity and directness which is lacking in some of the new illustrations. Figure 2-1 has been reduced too far: the inches and centimeters are only 80 per cent as long as they should be. The typography is pleasing.

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Photomicrography

Roy M. Allen, Fellow and Past President, The New York Microscopical Society. 2nd ed. D. Van Nostrand Co., Inc., Princeton, N. J., 1958. xiii + 441 pp. 235 figs. 54 plates. 16 × 23.5 cm. $9.

This second edition includes many of the developments in instrumentation which have occurred during the 17 years since publication of the first edition. The author comments on most of the instruments commercially available at the present time. Pictures of these instruments are also included. Microscopes, illuminators, cameras and photomicrographic items are all included in the discussion on equipment. The discussion on the self-contained "universal" units is especially interesting and helps to clarify the large number of things to consider before purchasing such a unit. Each has its own advantages and strong points, depending on ultimate use. For handy reference purposes there are also included photographic chemicals and accessories; much of this latter information is not particularly new and would appear to be similar to information offered by the manufacturers.

Probably one of the most valuable contributions in the book is a collection of photomicrographs, all of which were taken by the author. These pictures are nearly all printed one to a page with a brief history of each specimen, plus complete information on how the micrograph was taken, including optics and illumination employed, exposure, filters, staining (if any), photographic materials, and magnification.

The information on photomicrographic technique which appears to be the essential reason for writing the book makes it of value as a teaching aid or reference book. While some of the contents are not new they are presented in such a manner as to be of value to most microscopists doing photomicrography. Such discussions as "common faults of photomicrography" and others dealing with light sources, magnification, fuzzy fields, and exposure, make this edition a practical working microscopist's handbook. The usefulness of photomicrography is emphasized by the many micrographs used to illustrate the book.

The various types of optical microscopes include polarizing, phase, dark field, metallography, interference, fluorescent, and ultraviolet. In addition, a short chapter on the electron microscope is included. While this chapter on the electron microscope does familiarize the reader with such instruments, it is unlikely that the practicing electron microscopists can find much of interest.

Not only will the amateurs gain from the section on homemade equipment but the professional may also obtain some useful ideas. The author fully understands the problem associated with the high cost of equipment which faces nearly all amateurs. It gives many useful and practical suggestions on how to construct equipment.

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Reviewed in this Issue

G. Brooks King and *William E. Caldwell*, The Fundamentals of College Chemistry

Roy M. Allen, Photomicrography

Thomas K. Sherwood, Mass Transfer Between Phases

Fernand Lot, Radioisotopes in the Service of Man

William H. Hamill and *Russell R. Williams, Jr.*, Principles of Physical Chemistry

Lillian Hoagland Meyer, Introductory Chemistry

Oscar E. Lanford, Using Chemistry

The Biological Replication of Macromolecules

Melville L. Wolfrom and R. Stuart Tipson, Editors, Advances in Carbohydrate Chemistry. Volume 13

Francis A. Warren, Rocket Propellants

Nathan Feifer, Adventures in Chemistry

Mass Transfer Between Phases

Thomas K. Sherwood, Prof. of Chemical Engineering, MIT. Phi Lambda Upsilon, Department of Chemistry, Pennsylvania State University, University Park, Pa., 1959. xi + 86 pp. 23 figs. 21.5 × 27.5 cm. Paperbound. $2.75.

The old master does it again. These notes result from the 33rd annual Priestley lectures delivered by Professor Sherwood at the Pennsylvania State University, April, 1959.

This presentation, although only 80 typewritten pages in length, is a brilliant review of the present knowledge of mass transfer between two phases. The discussion in this series has been restricted to phenomena occurring at small areas of contact between phases, since the object was to review the present understanding of the

mechanism of the transfer process. Any chemical engineer who let the field of mass transfer slip from his grasp during the past years will appreciate this review to bring him up to date in this important field. Even those who are familiar with this field will enjoy Professor Sherwood's method of presentation.

The subjects covered are as follows: molecular diffusion, diffusion coefficients in gases, diffusion coefficients in liquids, eddy diffusion, transfer from a solid to a turbulent stream, generalized "analogy," some of the better-known "analogies," the Chilton-Colburn analogy, compressible flow, mass transfer with chemical reaction, the "film" or Hatta Theory, the penetration theory, chemical reaction in a turbulent boundary layer, ion diffusion, suspended catalysts, fixed-bed catalysts, diffusion in porous catalysts, mass transfer between two phases, and barriers at phase boundaries.

This presentation should be on every chemical engineers' work shelf.

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Radioisotopes in the Service of Man

Fernand Lot. UNESCO, Paris, 1958. 82 pp. 13.5 × 21.5 cm. Paperbound. $1. (Available from UNESCO Publications Center, 801 Third Ave., New York 22.)

This publication is a small paperbound pamphlet (#XVI) in a UNESCO series dealing with various programs of this organization. It might be characterized best by saying that it has the highest density of isotope applications of any volume with which I am familiar. It is evident that it is not possible to treat in any detail most of the applications mentioned in the book, but it is of quite considerable usefulness in referring to a wide variety of isotope applications.

The volume is written with a somewhat popularized approach, although it maintains a good technical level throughout most of its pages. The first four chapters are general statements having to do with the era of radioisotopes, natural radioactivity, the production of radioactive isotopes (in the neutron furnace), and the detection of radiation.

Chapter V combines applications in physics, chemistry, biology, and agronomy; the following chapters have to do with the therapeutical use of radioactive isotopes, industry, and a potpourri ranging from the fight against insects to the study of meteorites. Chapter IX contains a few pages on the effects of radiation on living beings, with the last chapter being comments relative to the 1957 UNESCO Conference on Radioisotopes.

In general, it can be said that the author attempted to balance not only the use of isotopes in various fields, but also to ballance the work being done in various countries. Most of the applications are not identified as to where or by whom the work is being done, although some specific references are made to work in both eastern and western countries. The references made by country do not seem to refer to particularly typical or outstanding work, but rather seem to be an attempt to make certain that most of the major countries are mentioned somewhere in the book. There are a number of diagrams and four double pages of pictures, likewise distributed geographically. The book also contains a three-page glossary of standard terms.

The booklet contains a few errors. One reference is to the gas diffusion isotope separation in which the pamphlet states that a mixture of isotopes passes through a wall which tends to retain the lightest isotope. There is also a reference to GM counters which are reported to count beta or gamma radiation at rates up to 10,000 per second. A reference is also made to "Professor Galvin's" work on photosynthesis. There also seems to be some uncertainty about the definition of a biological half life. On the whole, there are remarkably few such errors, and likewise only a few typographical errors.

This pamphlet would probably be of considerable use as a rapid, semitechnical introduction to the use of radioactive isotopes in any situation in which such a survey would be useful. Since no substantiating information is given other than the statement that a certain type of work is or has been done, it would not be particularly valuable as a research reference. It appears to have quite useful possibilities for the casual reader with some technical background.

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Principles of Physical Chemistry

William H. Hamill, University of Notre Dame and *Russell R. Williams, Jr.,* Haverford College. Prentice-Hall, Inc. Englewood Cliffs, New Jersey, 1959. x + 607 pp. Figs. and tables. 16 × 23.5 cm. $8.75.

This book is addressed to the student who can think, or who is willing to try to learn to think. It will be necessary, often, to read a sentence more than once, to diligently study a table or a graph. But once understood, there will be no need to learn a different interpretation in future studies. There are no tacitly qualified statements in this text. The authors are to be commended for their careful choice of precise, informative, and accurate statements. This text is outstanding for the rigor of its treatment.

The material that should be discussed in an elementary treatment of physical chemistry is growing daily. For this reason, perhaps, the authors have chosen to discard the traditional order of presentation. This choice has enabled them to include the necessary new additions without extensive digressions that would be forced into the older, classical, order of discussion. After an introduction consisting of an accurate, ordered choice of preliminary topics, the First Law is explained in Chapter 2. This is followed by a discussion of thermochemistry and the Second Law. In Chapter 5 the free energy equation is derived, and with this important matter now in hand, entropy, the Third Law, various types of equilibria, and electrochemistry are treated in the following six chapters.

Chapter 12 consists of a thorough, modern, treatment of chemical kinetics. A discussion of colloids and surface chemistry follows, notable for its attention to the mathematical details associated with this topic. In the remaining third of the book emphasis is placed upon chemical physics, beginning with atomic structure, continuing through nuclear chemistry, molecular structure, the solid state, photochemistry, and radiation chemistry. (As might be expected from the research interests of the authors, this chapter will be stimulating to the students.) The final chapter is on chemical statistics.

There are three features in this text that indicate it was written for the student. First, within each chapter are several exercises clearly designed to help the student understand the material discussed in the chapter. In general, the exercises are simpler than the problems at the ends of the chapters and thus they also prepare the student to meet the challenge of the problems. Second, at the end of each chapter the material is briefly summarized. Third, there are frequent references to the literature throughout the text and a selected bibliography in an appendix lists several other texts and reference works.

A reviewer, in order to convince the reader of his objectivity, should find a few things wrong with a text under review. No doubt this text has its flaws but they are not very obvious. Perhaps some will decide that the rigorous style of writing will not inform the student as well as has been implied here. I would have liked to see several references, perhaps as a short bibliographical listing at the end of each chapter, to the many excellent articles that have appeared in J. CHEM. EDUC. from time to time. But these are minor points. This text is recommended for serious consideration to all who are looking for a different text for the physical chemistry course. If not used in the classroom, it should be available in the library. It will also serve as a concise summary of modern physical chemistry for others who wish to refresh their understanding.

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Introductory Chemistry

Lillian Hoagland Meyer, Western Michigan University. 2nd ed. The Macmillan Co., New York, 1959. viii + 528 pp. Figs. 14.5 × 22 cm. $6.

There is great similarity in subject matter between this second edition and the first one which appeared eight years ago. The book is of approximately the same length. The chapter headings are alike. The material is directed toward the same group of students in home economics, nursing, physical education, agriculture, and biology. However, the text has been brought up-to-date by additions and sub-

(*Continued on page A704*)